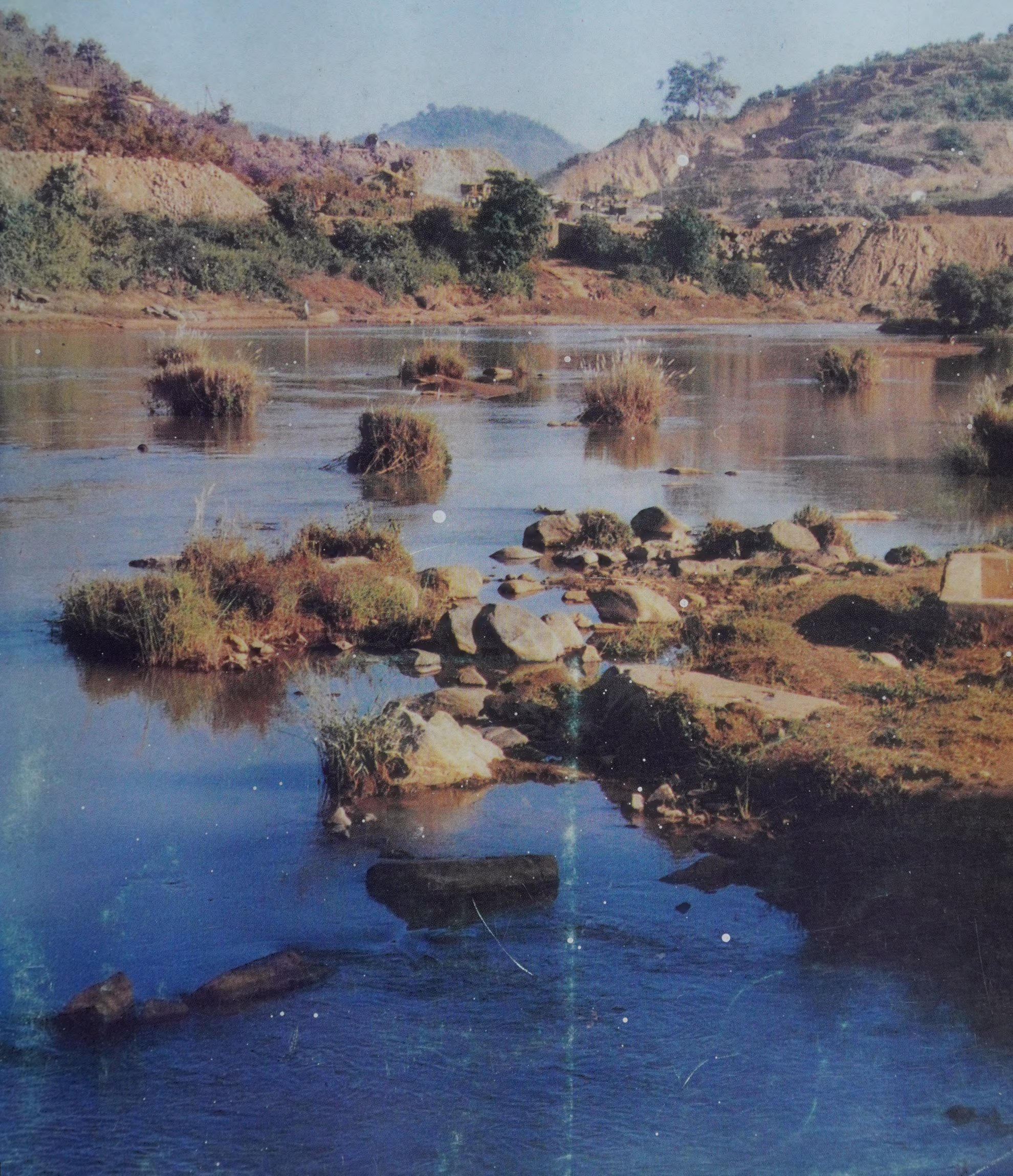
VECTOR CONTROL RESEARCH CENTRE

ANNUAL REPORT - 1988 (April to December)



WORLD HEALTH ORGANIZATION
Collaborating Centre for Research & Training in
INTEGRATED METHODS OF VECTOR CONTROL

FRONT COVER:

The river Muran in Koraput Dt., Orissa. *Anopheles fluviatilis* which breeds in this river, has been found positive for both oocysts and sporozoites (Section 2.1.4–Malaria studies).

BACK COVER:

A 100 litre capacity bioreactor has been installed at the VCRC which will be used for fermentation of biocides and cyclosporin. (Section 4.7 and 4.12–Biological Control).

VECTOR CONTROL RESEARCH CENTRE

PONDICHERRY 605006
INDIA

ANNUAL REPORT
1988
(April to December)

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This report covers the period April to December 1988, as the I.C.M.R now wants the annual reports for calendar years, and, not for financial years. The report for 1987–88 published last year already covered work done from January to March 1988.

CONTENTS

I. PREFACE

During the period under review the Centre continued to plod in keeping the scientific activities at a high tempo. A significant development is that the Koraput field programme on malaria studies and the M.Sc. (Medical Entomology) programme have been included under intramural plan activities of the Centre and have thus become permanent activities of the Centre from April 1988. There is no more ad hocism on the running of these programmes. The Shertallai programme on the control of *Brugia malayi* vectors through community participation continues to be under the specially funded Science and Technology mission project. The term of the Bangalore Mosquito Control Project is due to end in March 1989, and a master plan for mosquito control in Bangalore City, submitted in February 1988, is going through numerous committees constituted by the Government of Karnataka. There is no more excuse for not doing the job, now that they have a plan with all breeding places marked. The VCRC team will be shifted from Bangalore and posted elsewhere. In the ultimate analysis, no one in authority really wants to undertake organized vector control or urban mosquito control. While one may feel frustrated, we could draw solace from the experience of the legendary William Gorgas, who controlled yellow fever in Havana, Cuba. The Panama Canal Commission in 1904, would have nothing to do with Gorgas and "were determined to get rid of him as soon as possible. The enthusiasm of Gorgas for controlling mosquitoes was openly rediculed and his activities were repeatedly curtailed" (Paul Russel: Man's Mastery of malaria).

The Scientific Advisory Committee (SAC) of the Centre met on 29th April 1988 and the meeting was attended by (1) Dr. P.K. Ramachandran, Director of Defence Research and Development Establishment, Gwalior, (2) Dr. S.P. Tripathy, Additional Director General of ICMR, (3) Mrs. Uma Pillai, Senior Deputy Director General of ICMR, (4) Mr. B. Bhattacharya, Financial Adviser of ICMR, (5) Dr. L. N. Mohapatra, Director of Regional Medical Research Centre, Bhubaneswar, (6) Dr. M.K.K. Pillai, Professor of Zoology, Delhi University, (7) Dr. N. Ramakrishnan, Professor of Entomology, IARI, Delhi, (8) Dr. O.P. Bharghava, Dean, Sri Ramachandra Medical College, Madras, (9) Dr. V. Sambasivam, Retd. Director of Health, Pondicherry, and (10) Mr. V. Krishnamurthy, Health Secretary of Kerala Govt.

From the academic year 1988–89 onwards, the Indian Council of Medical Research is paying Rs. 800/- per month to the M.Sc. (Medical Entomology) students selected through open competition, as stipend, on the recommendation of the SAC.

The Health Secretary of Kerala agreed to the recommendation of the SAC to take up the implementation of the engineering solution to the Alleppey Canal for control of vectors of *Wuchereria bancrofti*.

Another recommendation of the SAC was to initiate field trials with *Ivermectin* against *Brugia malayi*. In this connection a team consisting of Dr. C.P. Ramachandran, Secretary to Steering Committee on Filariasis, Dr. P. Ranque, Chief, Filariasis of WHO, visited Alleppey/Shertallai from 6–8 November and held discussions with Prof. R.K. Shenoy, Professor of Medicine, Alleppey Medical College to carry out a phase II trial. A proposal is under preparation for submission to the SC/Fil for funding.

WHO/TDR Meeting at Pondicherry 19-28 October 1988:

The Informal Consultation on Bacterial formulations for cost effective vector control in endemic areas was held from 19–21 at the VCRC and which was followed by a meeting of the Steering Committee on Biological Control of Vectors from 24–28 October 1988. The meetings were organized by the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases of WHO, Geneva. The meetings were attended by 25 scientists, five from India and the remaining 20 from other countries.

Institutional Strengthening Grant:

The Centre was the recipient of a five year Research Strengthening Grant from the WHO/TDR programme, from 1983–1987. The grant was intended to improve the research, training and educational capabilities of VCRC in the epidemiology and control of vector borne diseases with special emphasis on the development of biological control of vectors. A team consisting of Dr. M.S. Mulla, Professor of Entomology, University of California, Riverside (Chairman), Dr. H.C. Chapman, Executive Director, American Mosquito Control Association, USA, Dr. Rene Le Berre, TDR, WHO, Geneva and Dr. B. Dobrokhotov, Secretary, Steering Committee/Biological Control of Vectors, WHO, Geneva, evaluated the work and progress achieved under the Institutional Strengthening Grant, on 23rd October 1988.

Linkages:

One of the most fruitful collaborative arrangement is with Dr. D.A.P. Bundy, Director, Parasite Epidemiology Research Group, *Imperial College, University of London* and his team consisting of Dr. A.M. Cairncross of the London School of Hygiene and Tropical Medicine, and Dr. B.T. Grenfell, of the Parasite Population Ecology Group of University of Sheffield, U.K. Parasite epidemiology is relatively a new field in India and collaborative arrangement with Dr. Bundy's group helped in providing advanced training to the VCRC staff in this new area of research. Voluminous data have been gathered during the 5 year Filariasis Control Demonstration Project and currently in the Science and Technology Mission Project on control of *Brugia malayi* vectors. Such data have been analysed in different ways with a view to developing population dynamics models of filariasis transmission to assist the future planning of control strategies. The visit of Dr. Bundy and his team has been approved by ICMR and is funded by Wellcome Trust. Preliminary analyses are presented in this report. It is hoped, in due course, the VCRC would develop adequate expertise in this field.

The Centre has close collaboration with the Defence Research and Development Establishment (DRDE), Gwalior. This organization had helped in the purification and identification of fungal metabolites produced by the VCRC. The DRDE also provided training to VCRC staff in immunochemistry and HPLC.

The VCRC continues to be a WHO Collaborating Centre for Research and Training in Integrated Vector Control. The UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases and the Division of Vector Biology and Control of the World Health Organization, Geneva, have assisted the Centre in many ways. This is a contributing factor for the rapid advancement of VCRC in several areas.

Acknowledgement:

That a large volume of quality work was produced, in spite of limitations of staff, speaks of the dedicated work of the scientists, who also shouldered a heavy teaching assignment for the M.Sc (Med. Ent) programme, and the discipline and work ethic of all the other staff. I would like to express my gratitude to them.

31 Jan 1989.

DR. P. K. RAJAGOPALAN
Director

II. STAFF POSITION AS ON 1st JANUARY 1989

DIRECTOR		Dr. P.K. Rajagopalan, M.Sc., Ph.D., M.P.H. (Calif.)
DEPUTY DIRECTOR	:	Dr. P.K. Das, M.Sc., Ph.D.
ASSISTANT DIRECTOR	:	Dr. K. Balaraman, M.Sc., Ph.D. Dr. K.N. Panicker, M.Sc., Ph.D. Dr. S.G. Suguna, M.Sc., Ph.D. Dr. S.P. Pani, M.D.
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RESEARCH OFFICER /	:	Mr. N. Arunachalam, M.Sc.
SENIOR TECHNICAL OFFICER	:	Dr. T. Mariappan, M.Sc., Ph.D. Dr. K. Krishnamoorthy, M.Sc., Ph.D. Mr. S.L. Hoti, M.Sc., Mr. K. Gunasekaran, M.Sc. Dr. Lalit Kumar Das, M.B.B.S. Dr. A. Sudhakara Rao, M.B.B.S. Dr. (Miss) P. Goverdhini, M.B.B.S.* Dr. (Mrs.) Prathibha Jayasimhan, M.B.B.S.* Dr. S.S.S. Mohapatra, M.B.B.S.** Mr. M. Kuppusamy, M.Sc.
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TECHNICAL OFFICER	:	Mr. K.D. Ramaiah, M.Sc. Mr. D. Dominic Amalraj, M.Sc. Mr. N. Pradeep Kumar, M.Sc. Mr. G. Rajendran, M.Sc. Mr. K. Viswam Mr. N. Somachary Dr. Raman Velayudhan, M.Sc., Ph.D. Mr. K.P. Paily, M.Sc. Mr. A.R. Rajavel, M.Sc., M.Phil. Mr. R. Srinivasan, M.Sc. Mr. S. Subramanian, M.Sc. Mr. N. Balakrishnan, M.Sc. Mr. P. Vanamail, M.Sc. Mr. Sarat Kumar Parida, M.Sc.
RESEARCH ASSISTANT	:	Miss V. Vasuki, M.Sc., M.Phil. Mrs. Nisha George, M.Sc. Mr. C. Sadanandane, M.Sc. Mr. Sudhansu Sekhar Sahu, M.Sc. Mrs. B. Nanda, M.Sc. Mr. R.S. Bhupathi, M.Sc. Mrs. Ambilikumar, M.Sc.

		Miss M. Jayasree, M.Sc. Miss R. Yasoda, B.Sc. Dr. P. M. Suresh Kumar, M.S.W., Ph.D. Mr. M.P. Prasad, M.Sc. Mr. V. Vijayan, M.Sc. Mr. Kailash Prasad Patra, M.Sc. Miss Githa Rani, M.Sc. Miss R. Shanthi, M.S.W.* Miss Abida, M.Sc.* Mrs. K.S. Snehalatha, M.A.* Miss A. Krishnakumari, M.A.*
STATISTICAL ASSISTANT	:	Miss A. Srividya, M.Sc. Mr. A. Manoharan, M.Sc.

ADMINISTRATION & ACCOUNTS

ADMINISTRATIVE OFFICER	:	Mr. N. Prem Kumar, B.Com.
ACCOUNTS OFFICER	:	Mr. S. Swaminathan
SECTION OFFICER	:	Mr. S. Chandrasekaran, B.Com.
SUPERINTENDENT	:	Mr. V. Vijayamoorthy, B.Com.
LIBRARIAN	:	Mrs. Sundarammal Rajendran, B.Sc., B.Lib.

* Project Staff
** Seconded by RMRC., Bhubaneswar.

III. RESEARCH STUDIES

1. SCIENCE AND TECHNOLOGY MISSION PROJECT TO CONTROL VECTORS OF MALAYAN FILARIASIS IN SHERTALLAI TALUK, ALLEPPEY DISTIRCT, KERALA STATE.

1.1. Precontrol epidemiological data.

Of the vector borne diseases lymphatic filariasis is a major public health problem in India. The Vector Control Research Centre (VCRC) is involved in the developement of appropriate strategies for the control of lymphatic filariasis in South India. One of the major activities of VCRC has been the control of Bancroftian filariasis but in 1986 a new programme was initiated to control Brugian filariasis. This technology mission project aims to control disease transmission in the Taluk (a sub-division of a District) of Shertallai in Kerala State, South India. The extent of the filariasis problem in this Taluk is indicated by the fact that the elephantoid swelling of the legs due to filariasis is commonly referred to as "Shertallai leg".

The pattern of transmission of Brugian filariasis is different from that of Bancroftian filariasis. The *Mansonia* mosquitoes (*M. annulifera, M. uniformis*), the vectors for Brugian filariasis, breed in hydrophyte infested water bodies which are predominantly found in rural areas. Hence the disease distribution is predominantly rural in contrast to Bancroftian filariasis, where the distribution is associated with the predominantly polluted water breeding sites of the *Culex* vector in urban areas. The VCRC has adopted an Integrated Disease Vector Management (IDVM) strategy to control Brugian filariasis based on environmental manipulation to reduce mosquito breeding, and treatment of parasite carriers. The progress of these activities has been documented in earlier reports. The epidemiological features of endemic Brugian filariasis in Shertallai as determined by pre-control surveys are presented below. Shertallai Taluk is divided into 18 administrative areas called Panchayats. The programme study area included 11 Panchayats covering a total population of 158,700 (1986 population estimated from 1981 census).

A peripheral blood microfilaria survey and a clinical survey were conducted during February to August 1986, to determine the pre-control epidemiological situation. Since the population is not uniformly distributed, a stratified random sampling protocol with proportional allocation of 10% and 3% of the population of each Panchayat was adopted for the microfilaria and clinical surveys, respectively. The programme was designed to collect blood smears from approximately 15,900 persons (10% of the total population) and clinically examine 4800 persons (3% of total population) in the study area. The samples in both the surveys were age stratified and weighted according to the demography of the area, with a target of a minimum of 5% and 2.5% in each age class in the microfilaria and clinical surveys, respectively. Households for sampling within each of the 11 Panchayats sampled were identified by using random number tabies.

On the day before the surveys a social worker visited each household and informed them of the details of the project and recruited people for the study. The blood collection teams visited the households between 20.00 and 24.00 hours and a 20 mm^3 peripheral blood smear was collected from each individual for subsequent laboratory assessment. All microfilaria carriers detected, were treated with DEC (at the dosage of 6mg/kg of body weight for 12 days) and thereafter followed in the VCRC Filariasis clinic situated at Shertallai town. For the clinical survey a team of physicians accompanied by sociologists visited the households and examined all available persons for filarial manifestations in addition to enquiring about the clinical history. Treatment was offered for any clinical condition diagnosed. The clinical cases of filariasis were also referred to the VCRC Filariasis clinic for follow-up. For both the surveys permission was obtained from all individuals, or in the case of children, their guardians.

A total of 22,369 blood smears (14.1% of the total population) was collected and examined for microfilaraemia. In the clinical survey 7,197 persons (4.5% of total population) were examined for filarial disease manifestations. The age stratification of the samples in both the surveys resembled the age-distribution of the population with the exception of the 0–5 years age-class in the

clinical survey which was slightly under-target sample size (Fig. 1.1., Table 1.1).

Overall patterns of infection and disease:

The prevalence of microfilaraemia increased monotonically in both sexes in childhood and throughout the young adult age-classes to attain a peak approximately at 20 years of age (Fig.1.2). The infection prevalence declined from 20 years to approximately 35 years of age and thereafter remained relatively stable throughout adulthood. Comparison between the sexes showed a significant difference only in the age-range of 25–34 years (Table 1.2), with infection being less prevalent in females.

The age-intensity of infection was measured as mean microfilarial density, which also increased monotonically in both sexes from childhood until approximately 20 years of age. Above 20 years the intensity declined sharply until approximately 30 years and thereafter remained relatively stable (Fig. 1.3). In general, the age-intensity profiles in both sexes showed a similar trend as age-prevalence.

The frequency distribution of microfilarial counts showed a markedly overdispersed pattern (Fig. 1.4). While the microfilaria counts ranged between 1 and 140 per 20mm^3 blood, the mean infection intensity was 0.31 per 20mm^3 in the study population. The frequency pattern of microfilarial counts was not adequately described by the negative binomial probability distribution.

The prevalence of disease was measured by taking into consideration both acute and chronic manifestations of filariasis using standard diagnostic criteria. Of the 7,197 persons clinically examined 716 had definite clinical manifestations of filariasis accounting for a disease rate of 9.9%. The prevalence of disease was clearly age-dependent in both sexes (Fig. 1.5., 1.6). Disease prevalence was lower than infection prevalence until about 15 to 20 years, thereafter the disease prevalence showed a monotonic increase in both sexes while the microfilaria prevalence remained low and relatively stable. Comparison between females and males did not show significant differences in disease prevalence in any of the age classes. Chronic lymphodema of the lower limbs was the predominant clinical presentation in both sexes.

Geographic distribution of infection and disease:

Comparison of infection and disease prevalence in different Panchayats showed that the distribution of filariasis was not spatially homogeneous in the study area (Table 1.3). The Panchayats in the Western part of Shertallai Taluk adjacent to the Arabian sea showed a higher prevalence rate compared to the Eastern or Northern Panchayats. The pattern was similar for both infection and disease prevalence. Comparison of the age prevalence of infection in 7 of the 11 Panchayats showed considerable variation in the different areas (Fig. 1.7), although the age profiles of areas with significant prevalences of infection were qualitatively similar.

Comparison with previous studies:

Comparison of a series of studies from 1934 (Iyengar, 1938; Jaswant Singh *et al.* 1956 and Russel *et al.* 1976) to the present in 2 of the 11 Panchyats, showed that there has been a significant decline in the prevalence of both infection and disease over the last five decades (Fig. 1.8). When the study area was divided into three zones, to observe the geographical pattern of decline over the last fifty years, the results indicated that all three zones showed qualitatively similar change (Fig. 1.9). The results also indicated that the prevalence has remained highest in the Western zone throughout the period from 1934 to 1986.

The comparison of average microfilarial intensity among the infected individuals in 5 of the 11 Panchayats between 1955 and the present study also showed a significant decline (Fig. 1.10). Comparison of the age distribution of microfilaria and disease prevalence again showed a significant quantitative decrease, although the relationship between prevalence of infection and disease remained qualitatively similar in both studies (Fig. 1.5., 1.6., and 1.11).

The study confirms that Shertallai remains endemic for Brugian filariasis. The distribution of infection was not however homogeneous throughout the Taluk: the prevalence of microfilaraemia ranged between 0.11% and 6.94% in

TABLE 1.1

Age distribution of samples in microfilaria and clinical surveys in study population.

	MICROFILARIA SURVEY		CLINICAL SURVEY	
Age Groups (years)	Sample size	% of the Population	Sample size	% of the Population
0–4	1160	5.04	559	2.43
5–9	2098	8.81	809	3.40
10–14	2665	13.43	781	3.94
15–19	2686	19.45	712	5.16
20–24	2379	18.98	659	5.26
25–29	1807	15.39	576	4.90
30–34	1756	16.77	523	4.99
35–39	1573	16.52	441	4.63
40–44	1015	12.30	288	3.49
45–49	1214	18.21	342	5.13
50–54	974	16.59	316	5.38
55–59	955	26.16	298	8.16
60–64	925	22.42	319	7.73
› 64	1162	21.53	574	10.64
TOTAL	22369	14.10*	7197	4.53*

* Mean

TABLE 1.2

Age and sexwise infection and disease prevalence in study area.

	MICROFILARIA SURVEY				CLINICAL SURVEY			
Age Group (Years)	Male sampled	mF Rate	Female sampled	mF Rate	Male sampled	Dis. Rate	Female sampled	Dis. Rate
0	0	0	0	0	0	0	0	0.00
1–4	630	0.95	530	0.75	272	0.00	287	0.00
5–9	1063	1.22	1035	2.03	391	0.77	418	1.20
10–14	1352	3.25	1313	3.12	353	2.83	428	2.80
15–19	1159	3.80	1527	3.73	233	3.00	479	6.26
20–24	927	2.80	1452	2.55	171	2.34	488	5.53
25–29	716	3.07	1091	1.28	136	11.76	440	6.36
30–34	716	2.51	1040	0.96	125	8.80	398	9.05
35–39	679	2.50	894	2.24	141	14.18	300	9.33
40–44	440	2.50	575	1.22	87	11.49	201	12.94
45–49	493	2.43	721	1.39	95	11.58	247	14.57
50–54	424	2.59	550	1.64	110	17.27	206	21.84
55–59	430	2.09	525	3.05	91	20.88	207	24.15
60–64	426	2.35	499	1.40	115	23.48	204	25.98
›=65	562	2.14	600	1.50	222	34.23	352	30.40
TOTAL	10017	2.55*	12352	2.12*	2542	9.17*	4655	10.38*

* Mean

TABLE 1.3

The population and sample distribution according to geographical areas (Panchayats).

		MICROFILARIA SURVEY			CLINICAL SURVEY	
Area No.	Panchayat Name	Population	Sampled	% Coverage	Sampled	% Coverage
1	Arookutty	14080	1883	13.37	ND	ND
2	Perumbalam	8990	1116	12.41	638	ND
3	Kadakarapalli	16165	2208	13.66	638	3.95
4	Shertallai South	32921	6717	20.40	3990	12.12
5	Vayalar	20698	2612	12.62	607	2.93
6	Kuthiathode	18531	2248	12.13	553	2.98
7	Pattanakad	6223	752	12.08	112	1.80
8	Mararikulam	3510	389	11.08	154	4.39
9	Muhamma	14402	1748	12.14	315	2.19
10	Aroor	6970	698	10.01	233	3.34
11	Pallipuram	16210..	1998	12.33	595	3.67
TOTAL		158700	22369	14.10	7197	4.53

ND — Not Done; * = Mean

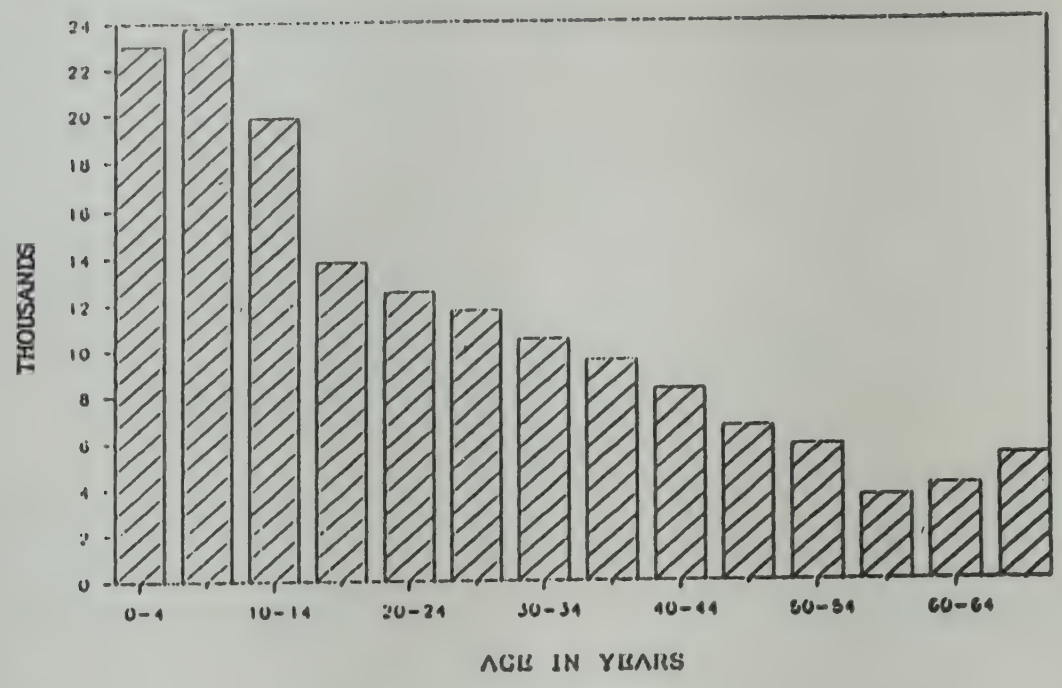


Figure 1.1 Age-Structure of population of study area

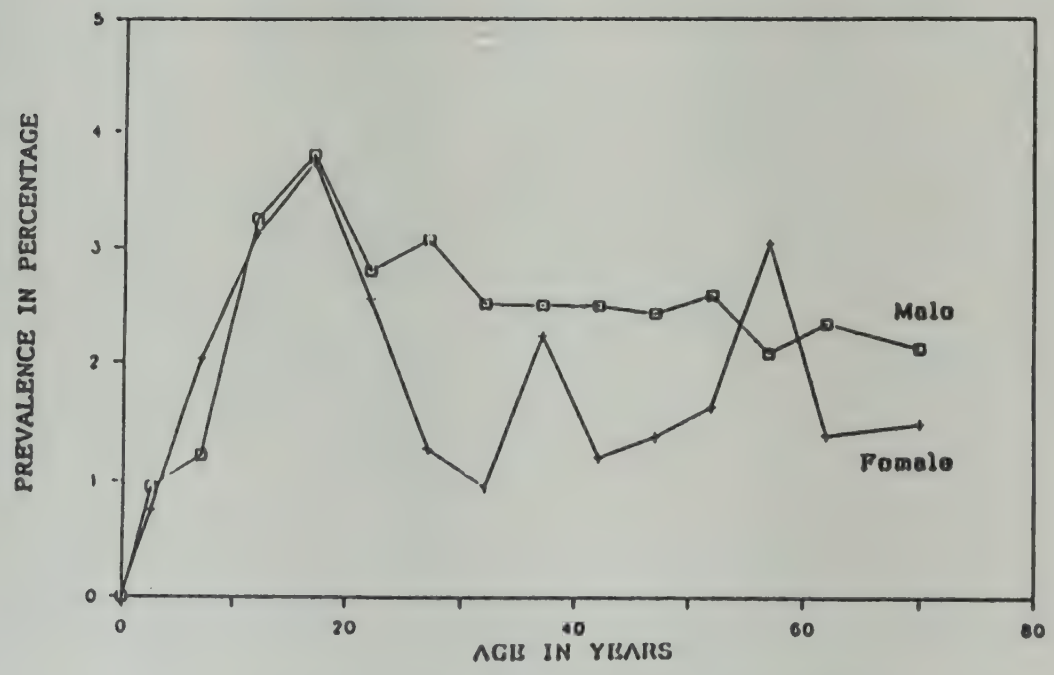


Figure 1.2 Age and sexwise prevalence of infection

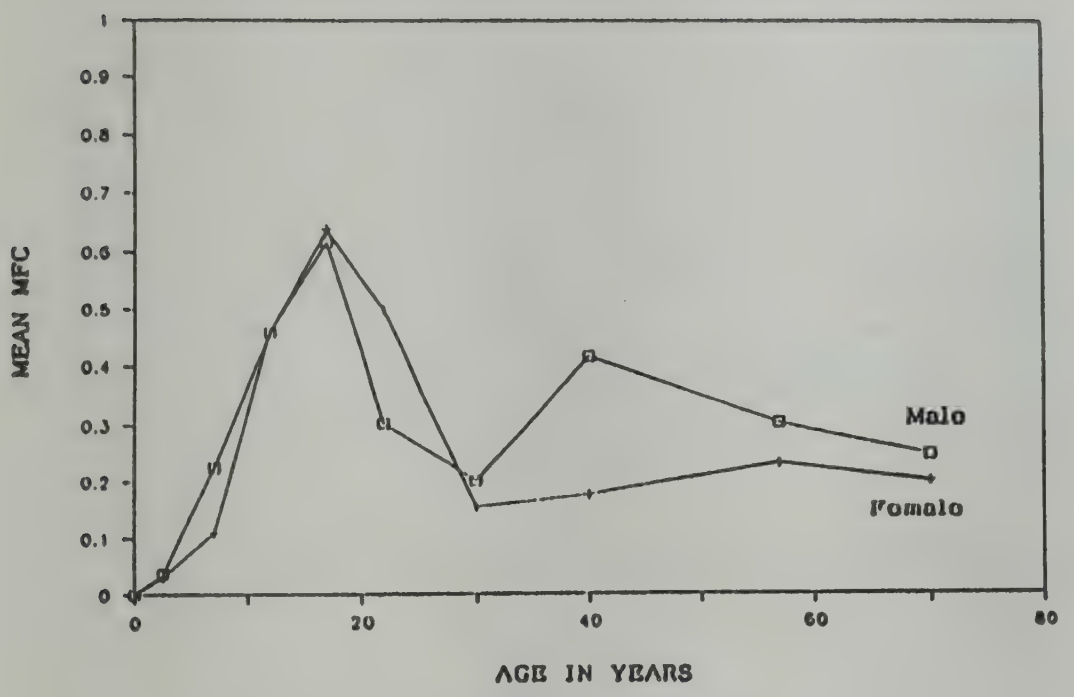


Figure 1.3 Age and sexwise intensity of infection

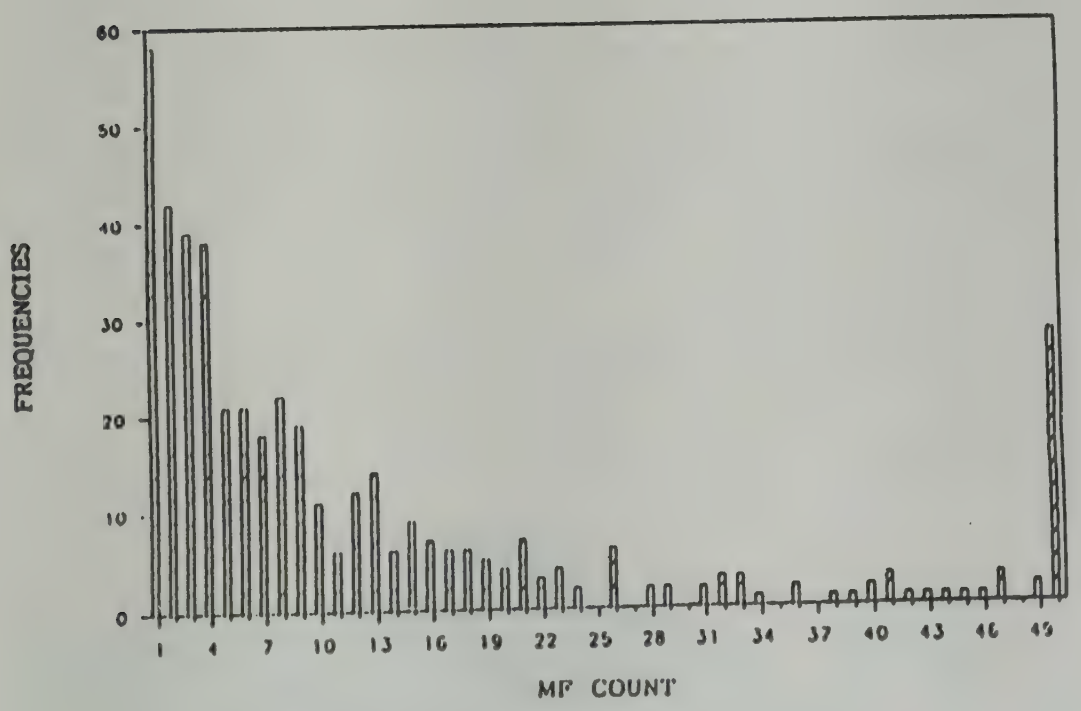


Figure 1.4 Frequency distribution of microfilaria counts in infected individuals.

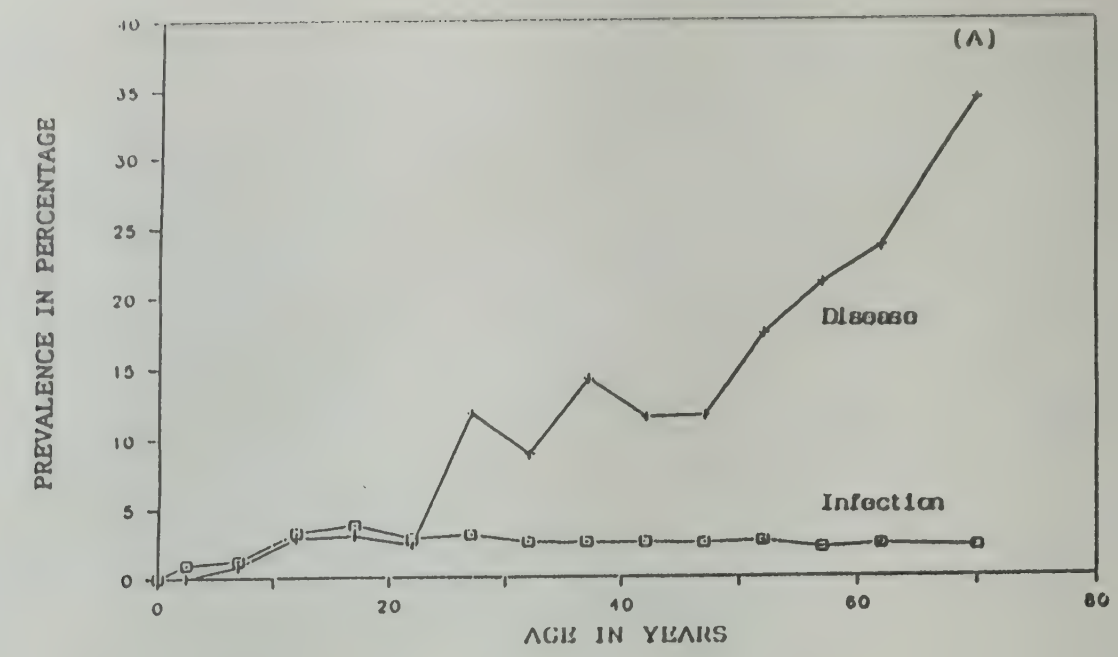


Figure 1.5 Agewise comparison of infection and disease prevalence in males.

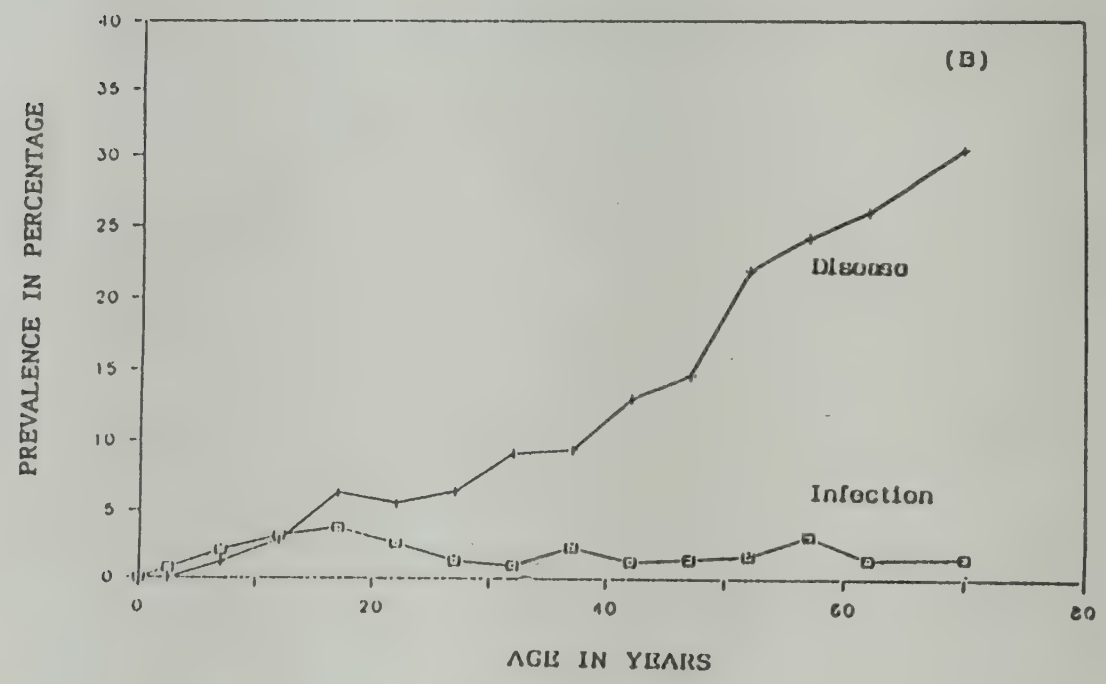


Figure 1.6 Agewise comparison of infection and disease prevalence in females.

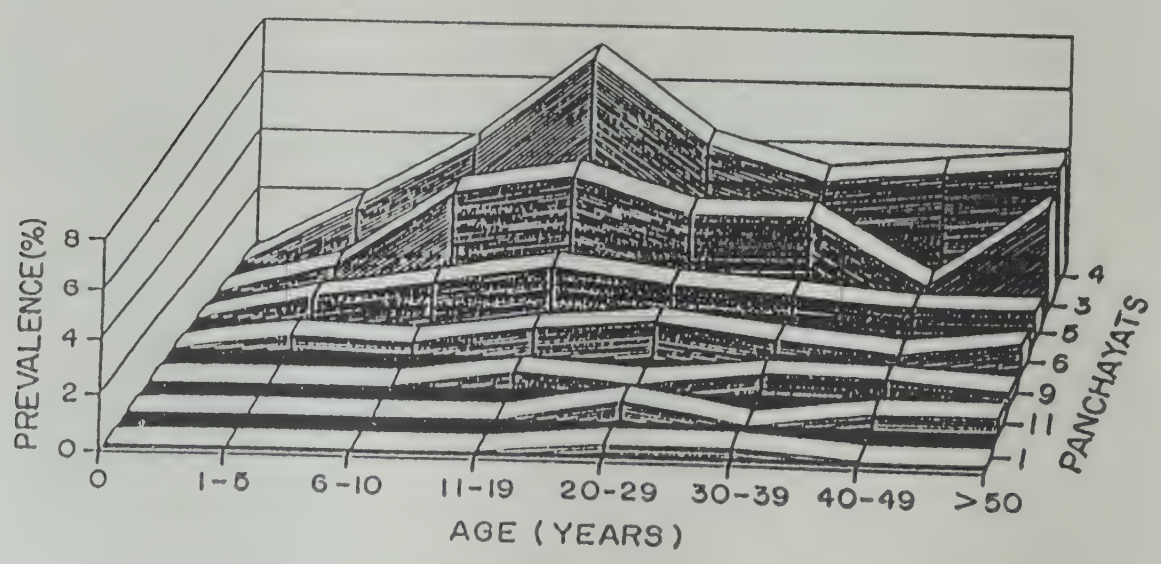


Figure 1.7 Agewise infection prevalence in selected Panchayats. (For Panchayat numbers, refer Table 3 and Figure 1).

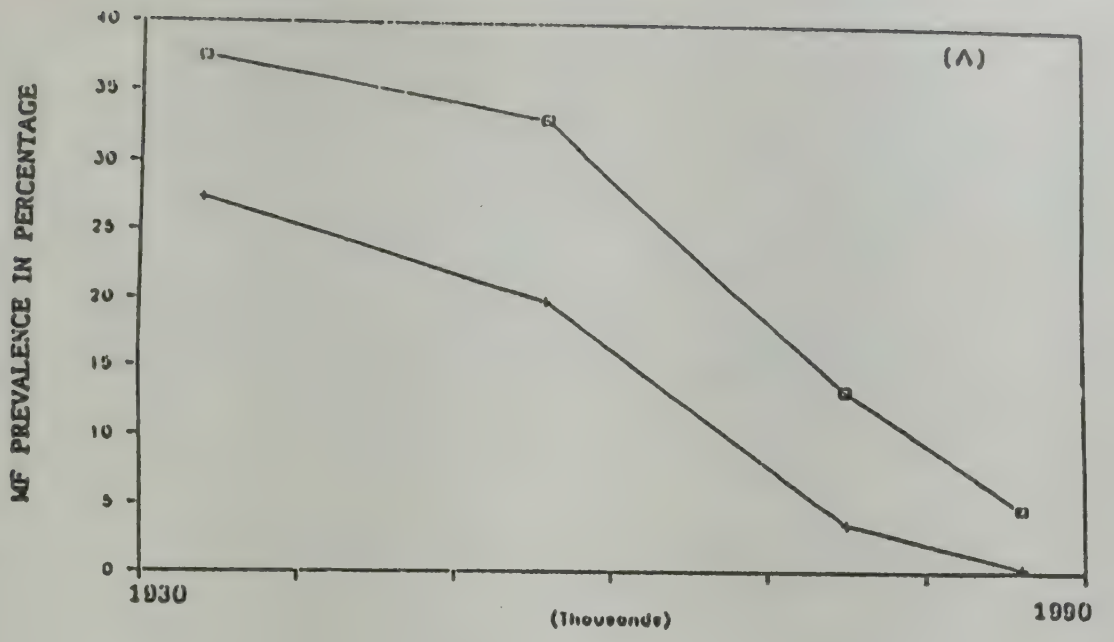


Figure 1.8 Pattern of change in infection (A) and disease (B) prevalences in two selected Panchayats between 1934 and 1986.

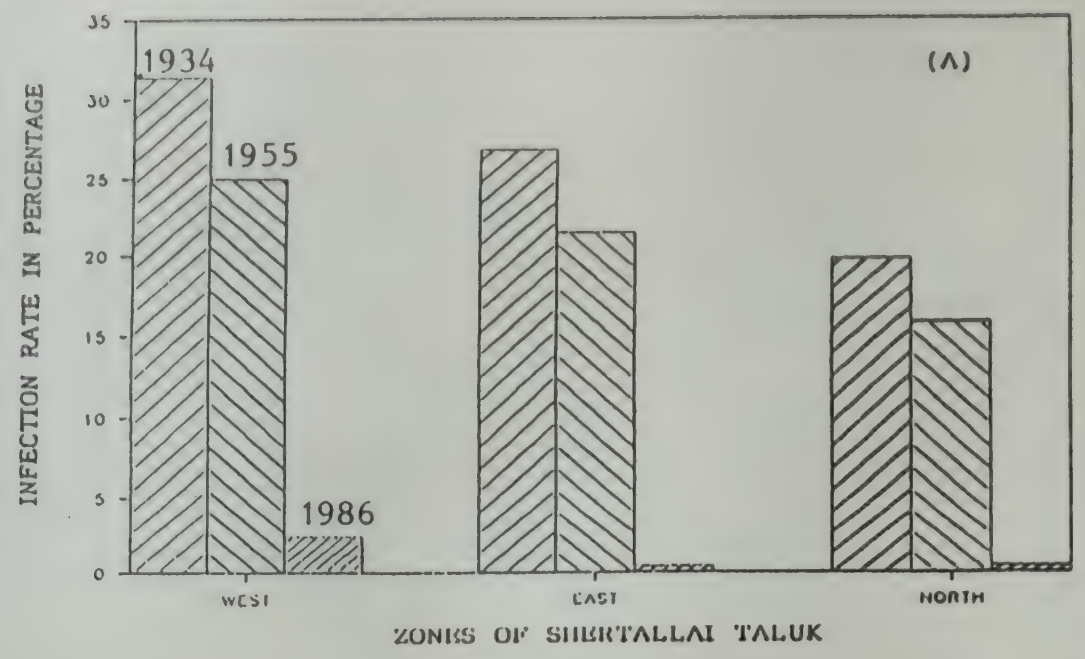


Figure 1.9 Pattern of change in infection (A) and disease (B) prevalences in three zones of study area.

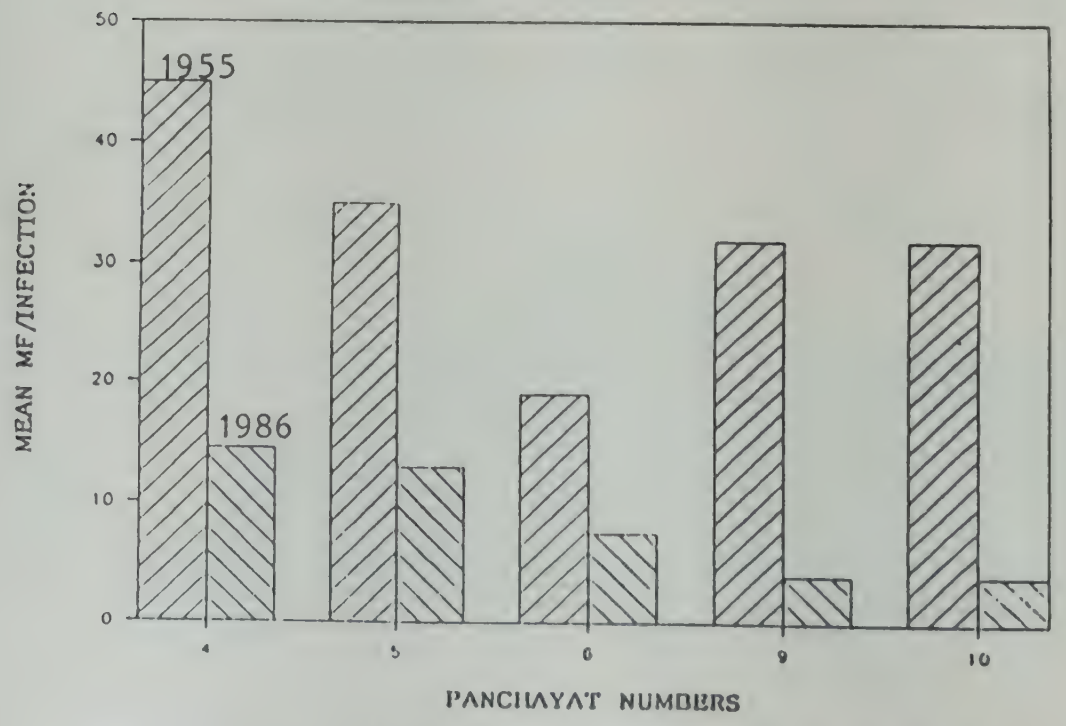


Figure 1.10 Pattern of change in mean microfilariae counts among the infected individuals in 5 selected Panchayats.

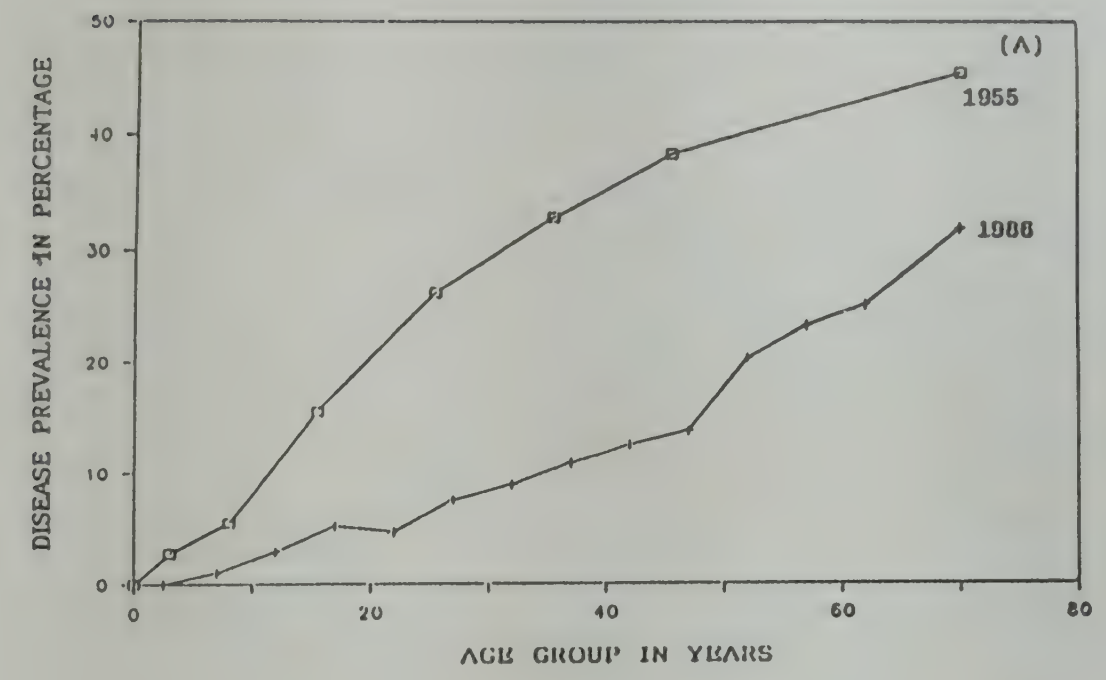


Figure 1.11 Pattern of change in agewise infection (A) and disease (B) prevalences between 1955 and 1986 in study area.

different Panchayats. The prevalence rates observed in the present study are likely to be an underestimate of the actual prevalence, as the peripheral blood smear examination technique employed lacks sensitivity. More sensitive techniques, such as filter concentration methods were not however considered suitable for the present mass survey application.

The pattern of age prevalence showed a qualitative similarlity with that observed for Bancroftian filariasis in Pondicherry. It is not known whether the observed age-dependency is due to the age-dependency of the acquisition of infection or the age-dependency of host resistance. The age intensity profile also resembled that observed for *W. bancrofti* in Pondicherry. It has been suggested that the age at which peak intensity of infection is attained is related to the parasite life span. The fact that this peak was around 20 years of age for both *B. malayi* (present study) and *W. bancrofti* in Pondicherry indicates that the life span of adults of both parasites may be similar in human hosts.

The frequency distribution of microfilarial counts also resembled that for *W. bancrofti* observed in an earlier study of VCRC in Pondicherry, with a larger proportion of microfilaria negative individuals than would be predicted, even when the sampling sensitivity is discounted. The epidemiological implications of this are under study.

The disease prevalence was higher than infection prevalence throughout the study area. It ranged between 3.17% and 14.29% in the different Panchayats, and the geographical distribution resembled that of infection prevalence. The analysis of rate of change in disease prevalence with age showed a monotonic age-dependent increase. As the chronic manifestations of disease are permanent (unless treated surgically), and as these were the predominant presentations recorded in the present study, the pattern of age prevalence suggests the accumulation of clinical cases with age.

Comparison of the results of the present study with earlier studies indicates a major decline in filariasis prevalence in the locality over the last five decades. The precise reasons for the decline in prevalence are not known. Several factors in combination might be responsible: introduction of DDT during the Malaria control operations initiated in 1959; introduction of di-ethyl carbamazine (DEC) in the late 1950s; improvements in socio-economic conditions; or enhanced awareness by the community in combination with increased availability of medicare. It has also been suggested that gradual replacement of *Pistia stratiotes* and *Eichornia speciosa*, the preferred hydrophytes of *M. annulifera*, by *Salvinia auriculata* could be responsible for a decline in vector density and hence infection prevalence.

These results indicated that despite a considerable fall in infection prevalence, the prevalence of disease in the Taluk remains unacceptably high (Fig. 1.11). The continuing high prevalence of disease is partly an historical feature: the older age classes represent persons who acquired infection and disease in previous decades when transmission also was high. It is also apparent, however, that conversion to disease is still occurring in the younger age classes (5% of 15 year olds have clinical filariasis) and thus, that current levels of transmission still have a significant clinical impact. It should be recognised in this context that the current level of infection prevalence as estimated by microfilarial positivity is an understimate, and the actual prevalence may be substantially greater. The aim of the VCRC programme is to hasten the eradication of disease from the Taluk, particularly the worst afflicted Western Panchayats, since without timely intervention it would appear that Brugian filariasis will remain a significant cause of ill health in some parts of Shertallai for many more decades.

1.2. Vector Management:

As already stated in earlier reports of the Centre (1986–87 and 1987–88), the main emphasis of the programme is to pass on the technology for vector management to the people of the area, among whom there is now an awareness that unless they themselves take an active part in carrying out vector control measures, the disease can not be eradicated from the Shertallai foci.

Composite fish culture has already been accepted by the community. Since last year, the Chinese grass carp (*Ctenopharyngodon idella*) has been added to the other species of fishes in com-

posite fish culture, and which has also been readily accepted by the community. The addition of Chinese grass carp had helped in preventing re-infestation of the ponds where it had been introduced. Out of a total of over 75000 ponds in this area, 35690 ponds have now been stocked with different fishes, including the Chinese Grass Carp so far. As already reported, composite fish culture is now being enforced by the volunteers of the FILCO (Filariasis Control) movement.

It is well known that no community participation will be sustained unless the community gets convinced of the benefits accruing to them. In a disease like filariasis, immediate gains are not easily perceptible to a largely rural population (unlike in malaria where disease control can be demonstrated). The people are more concerned with chronic manifestations of the disease rather than with microfilaria status. Therefore the necessity of incentives is all the more felt in Shertallai. The movement for composite fish culture has already gained momentum, but financial gains accrue only after a time. Therefore the VCRC has been looking for new avenues of enlisting the support and cooperation of other agencies—*intersectoral effort*.

1.2.1. Involving NABARD (National Bank for Agriculture and Rural Development):

Prompted by the VCRC, the NABARD has now come forward to assist the programme of composite fish culture with the State Bank of Travancore as the Lead Bank. Under its leadership, five other banks, located in Shertallai area have now joined the programme. The different banks are:

i. State Bank of Travancore
ii. Union Bank of India
iii. Canara Bank
iv. Corporation Bank
v. District Cooperative Bank
vi. Kerala State Cooperative Agricultural Development Bank

More banks are expected to join the programme.

The NABARD (which is a wing of the Reserve Bank of India) will be making financial allocation to the above named banks through the lead bank (State Bank or Travancore) for financing individual entrepreneurs for taking up composite fish culture in domestic ponds. The VCRC will be coordinating these activities through FILCO since fish farming will lead to elimination of vector breeding in ponds and ultimately to disease control.

The VCRC, through FILCO, has started identification of the beneficiaries and the banks will give loans to them at the rate of Rs. 1420 per pond. The loan is returnable in three years time. Nearly 2000 beneficiaries have been identified and their applications for loans are being processed by the banks concerned. Initially, the lead bank has allocated different localities to each of the constituent bank in the area. The NABARD will monitor how the scheme progresses and depending on the outcome, it is hoped, the NABARD will cover the entire Shertallai area, where *B. malayi* infection is prevalent. The beneficiaries will utilize the loan amount for preparation of the ponds like weed removal, building embankments, killing predator fishes like *Ophiocephalus* already present in the ponds, and for buying fish fingerlings for composite fish culture and fish feeds. Hitherto, the VCRC has been providing fish fingerlings free of cost. When anything is given free, there is not much value attached to it generally. Since they did not have any financial involvement, they were not much concerned with the returns. This trend is being corrected now, since the beneficiaries will actually take great interest in the pisciculture (hitherto looked after by the VCRC and FILCO) in a area where there was not much awareness or interest for inland fish culture. That this programme will succeed is confirmed by the large number of applicants for bank loans for fish culture.

1.2.2. Duck farming for weed control:

Currently, duck culture is being practised in a migratory manner in Kerala State, depending on the pattern of rice cultivation. Now stationary duck culture is being introduced by the VCRC. Two varieties, White Pekins and Khaki Campbell are known for their weed eating behaviour. At the instance of the VCRC, the State Bank of Travancore, is starting 20 units in Shertallai, with 25 birds each and these units will be entrusted to individual farmers in Shertallai on an experi-

mental basis. If successful, the scheme will be expanded. Apart from clearing of weeds by ducks, the scheme will also boost the economy of the local inhabitants.

1.3. Giant gourami:

As stated in last year's report, the Giant gourami is now being cultured by the VCRC in Shertallai because of its high weedivorous potential.

Eventhough this fish was very popular due to its large size, delicacy and weed eating behaviour, they are on the verge of extinction now. The heavy mortality of *hatchlings*, due to *Aeromonas* infection, frys and finderlings with *Argulus* and *Lernea* infestations coupled with delayed maturity resulting in harvesting the juveniles before attaining the reproductive age seem to be the major factors responsible for the depletion in their population. Moreover, cannibalism among the immatures and unfavourable environmental factors such as heavy rain, fluctuating water levels interfering with their nesting activities and absence of marginal vegetation for nidification in many natural habitats also are leading to extinction. A study has been initiated to assess the efficacy of these fishes in the control of hydrophytes. They prefer to devour *Pistia*, the favoured plants of *Mansonia* mosquitoes for breeding followed by *Salvinia* and *Eichhornia*. The daily consumption rate of these plants was found to be 206.25 $\pm$ 19.09 gm, 129.20 $\pm$ 5.14 gm. and 27.65 $\pm$ 5.00 gm/kg. body wt. respectively. The feeding rate was higher when all the three plants were offered together. No significant variation was observed in the feeding capacity both in laboratory (419.60 $\pm$ 28.05 gm) and in field (419.30 $\pm$ 36.70 gm). This confirmed the aquatic weed eating habit of this fish both in captivity and in nature. Further preliminary studies using this fish indicated that 122 to 180 numbers (144 to 168 kg/wt) are sufficient to clear habitats with a surface area of 10,000 sq.mtr. infested with weeds. Based on these results, field trials are started in 125 ponds at the rate of 12 fishes per 100m^2 of surface area.

1.4. Alternate source of green manure:

Growing of weeds in domestic ponds is greatly linked with the socio-economic life of the people in Shertallai. Eventhough, the weeds have very little manure value due to its abundance it formed a major green manure source for the people here. Hence, it was necessary to provide an alternative suitable green manure source in place of aquatic weeds. The legume plants such as-Sunhemp (*Crotalaria junca*) and Dhaincha (*Sesbania aculeata*) are well known for enriching the soil quality, besides their high manure value due to nitrogen fixation. While Dhaincha is more suitable for clayey soil, Sunhemp is ideal for sandy soil. These plants have been introduced by VCRC in Shertallai region as a substitute for weeds.

The legume seeds are distributed as an incentive for beneficiaries who are maintaining their ponds free from vegetation. So far, 62 kg. of Dhaincha and 103 kg. of Sunhemp seeds were provided to 292 households which resulted in making the people clear an area of 60,000 m^2 water bodies from weeds and thereby vector breeding.

The Agricultural Department, Government of Kerala, has also extended their full cooperation in popularising these legumes in Shertallai region.

1.5. Introduction of salinity for weed control:

Earlier observations in channels and canals of Shertallai showed that the hydrophytes do not grow when the salinity of the waterbody exceeds 1030 ppm. Taking advantage of this deleterious effect of salinity on the hydrophytes, attempts have been made to increase the salinity using common salt in polluted ponds, infested with weeds. Studies have been initiated to find out the effect of different salinity concentrations (500 ppm–3000 ppm) on various hydrophytes. Cost effectiveness of the programme will also be analysed subsequently.

1.6. Weed management through a mechanical device:

Alleppey-Shertallai Canal (A-S Canal) which passes through the heart of Shertallai Town is one of the largest water bodies of this region. The free floating weeds in the canal not only promotes vector breeding, but also obstruct navigation. The physical removal of weeds was not a practical proposition, since there is an influx of weeds from the adjoining Vembanad Lake. Therefore, a

simple floating device, was designed by the VCRC using buoyants and kept across the canal at the entry point near the lake. This system while preventing the influx of weeds into the canal, does not interfere with the plying of boats. A weed free canal helps not only to prevent the vector breeding but also enhace the navigational prospects.

1.7. *Neochetina:* a weevil infesting weeds:

Two species of *Neochetina* (*N. eichhornia* and *N. bruchi*) obtained from the Indian Institute of Horticultural Research, Bangalore, were utilized for mass culturing in the laboratory. For a field trial, 2000 weevils from the laboratory colony were released into a closed channel (surface area: 200 m^2, depth: 1 m) infested with *Eichhornia crassipes*. Monitoring on the propagation of weevils and damage of the plants, was made at monthly intervals. Six months after the release the weevils were established in the habitat as different developmental stages were observed. While 50% of the total plants were seen partly damaged, feeding scars were seen in almost all the plants. Further observations are in progress.

1.8. Entomological studies:

Adult vector population was monitored continuously in the Shertallai operational area as well as in the check zone, Mararikulam South (Ambalapuzha taluk). The average indoor resting density of Mansonioides (*M. annulifera*, *M. uniformis* and *M. indiana*) was found to be as low as 1.67/man/hour in the operational area while it was 6.07/man/hour in the check zone. Also, there was a drastic reduction in the average man biting rate in the operational area (MBR 18.55) when compared to check zone (MBR 37.11) indicating the effectiveness of various intervention measures adopted.

Analysis of abdominal conditions of Mansonioides mosquitoes resting indoors with a high fullfed to semigravid ratio (*M annulifera* 2.6:1. *M. uniformis* 4:1 and *M. indiana* 3.6:1) indicated their outdoor resting habits. Therefore, sampling of outdoor resting population of Mansonioides was carried out in places like bushes using sweep nets. Spending 64 man hours, 24 specimens of Mansonioides mosquitoes (*M. annulifera*—2 and *M. uniformis*—22) were collected. The abdominal status of *M. annulifera* was found to be unfed, while in *M. uniformis*, all different conditions (unfed—11, fullfed—5, semigravid—4 and gravid—2) were found. Sampling of a large population using other possible methods, to have a thorough understanding of their resting behaviour, is in progress.

The infection and infectivity rates were 0.35 and 0.12 respectively. In the check zone Mararikulam South, 574 specimens were dissected and the infection and infectivity rates recorded were 1.39% and 1.17% respectively. The reduction in the transmission evidenced in the operational area is indicative of the effectiveness of liquidation of parasite load following intensive mass drug therapy (see section 1.10).

Mansonioides mosquitoes breed in close association with aquatic weeds and their dependency on weeds for respiration is well known. Due to its obligatory association with weeds, they move rarely and hence, less accessible to conventional larvicides. In order to device an effective control measure, details on the attachment and detachment behaviour of these larvae are essential.

To begin with, larvae of mansonioides were obtained from the laboratory colony and exposed to the major host plants viz., *Pistia*, *Eichhornia* and *Salvinia* separately in plastic container with water. The larvae introduced in container were observed for 72 hours continuously. Observations indicated that they attached to the entire length of the roots in *Salvinia* (63.5%) and *Eichhornia* (70.0%) whereas in *Pistia*, only the growing parts of root and root caps (45.0%). Hardness of roots in *Pistia* and the tenderness in the case of *Salvinia* and *Eichhornia* may be the reason influencing the region of attachment. During the 72 hours of continuous observation, larval attachment and detachment was seen in 700 instances. Detachment from the root portions and leaf portions were observed in 503 and 197 instances and the reattachment to roots and leaf portions were 472 and 228 instances respectively. The time interval for detachment and reattachment varied from 30 seconds to one hour. In the absence of hydrophytes, early immatures survived relatively longer (192 hours), when compared to the late ones (64 hours). Higher metabolic activity resulting in increased oxygen requirement in late instars may be the reason for

their shorter survival. A similar pattern of movement (detachment and reattachment) of larvae seen in clear and polluted ponds rich in organic matter, further led to infer that the attachment behaviour is instinctive and not found to be influenced by food availability.

The propagation of hydrophytes and their invasion in newer water bodies are facilitated by their rapid vegetative growth. The rate of daily increase of *Pistia stratiotes*, *Salvinia molesta* and *Eichhornia crassipes* was studied assuming that each of them grow geometrically over a certain period. The relative growth rate (=% of daily increment) for *Pistia*, *Salvinia* and *Eichhornia* was observed to be 5–20%, 6–43% and 4–26% respectively, depending on the physico-chemical characteristics of the water bodies concerned.

1.9. Health Care Delivery:

Health Care Delivery is designed with the ultimate goal of eliminating the foci of filariasis transmission from Shertallai. Hence the avenues created under this component are aimed at parasitological and clinical detection of filariasis cases and imparting appropriate treatment. This is in addition to prevention of vector breeding. Different methods have been employed for this purpose, and attempts are made to benefit all strata of the community.

1.9.1. Filariasis Clinic at Centre:

Biweekly filariasis clinic continued to function at the field station. During this period, 2,467 patients attended the clinic and on an average these patients made 3 visits to the clinic. A majority (49.0%) had filarial manifestations.

Night blood collection for detection of microfilaraemia was carried out daily at the Centre. A total of 3,800 persons were examined and 36 (0.95%) microfilaria carriers were detected. All except two had *B. malayi* infection. *W. bancrofti* was detected in two persons and both of them had moved to Shertallai recently (less than 6 months) indicating that these are imported cases. All symptomatic and/or microfilaraemic cases were administered diethyl carbamazine (6mg/kg/day × 12 days). Other supportive treatment was imparted as per necessity.

1.9.2. Door to Door Survey:

Door to door clinical and parasitological surveys were carried out in a selected area in Mararikulam North Panchayat (population 625). This was done to collect pre control epidemiological data prior to initiating of monthly low dose mass drug administration (vide infra). A total of 571 (91%) and 604 (97%) persons were examined in the clinical and parasitological survey respectively. The disease prevalence was 6.3% and infection prevalence was 2.5%. The mean microfilaria count was 6.07 and MFD 50 was 3.26.

1.9.3. Filariasis Detection Camps: (FDC)

Night blood examination for microfilaria detection is also carried out through FDCs. These FDCs were organized by member organizations of FILCO, Student's Filariasis Control Clubs, Anganwadis of the *Integrated Child Development Services* (ICDS) and other voluntary organizations. In the FDCs organized by the Anganwadi's of ICDS emphasis was given to collect blood smears from children below 6 years age. This is necessary for early detection and treatment for prevention of morbidity.

A total of 83 FDCs were held during the period under report and 13,133 persons were examined. *B. malayi* microfilaria was detected in 190 (1.45%). The number examined included 1,409 pre school going children of whom 14 were microfilaria carriers. The FDCs are being held from October 1986 and a total of 147 FDCs have been conducted until December 1988. The panchayat wise results at blood examinations under FDCs is shown in Table. 1.4.

1.9.4. Health Camps:

Health Camps are organized in different parts of the taluk by the member organizations of 'FILCO'. Apart from detection of symptomatic and microfilaraemic cases, comprehensive health delivery is done in these camps. During the reporting period 5 such camps were held. A total of 3235 persons were examined. Clinical manifestations of filariasis was detected in 2.75% and macrofilaraemic cases in 2.39%.

The health camps were initiated during June 1986 and 16 such camps have been conducted

TABLE 1.4

Panchayat wise results of filariasis detection camps

Sl. No.	Panchayat Name	No. of FDCs	No. covered	No. + ve	mF Rate
1.	Shertallai South	29	4114	68	1.65
2.	Kadakarapalli	16	2245	39	1.74
3.	Shertallai Municipality	8	1893	10	0.53
4.	Pattanakad	10	1541	11	0.71
5.	Kuthiathod	2	266	1	0.38
6.	Muhamma	7	1278	4	0.31
7.	Thannirmukkom	16	2568	30	1.17
8.	Thykattussery	2	396	2	0.51
9.	Kanjikuzhi	5	758	9	1.19
10.	Pallipuram	3	502	0	0.00
11.	Thuravoor	6	670	4	0.60
12.	Mararikulam North	23	3711	144	3.88
13.	Vayalar	13	2091	15	0.72
14.	Panavally	1	163	3	1.84
15.	Ezhupunna	1	80	3	3.75
16.	Arookutty	2	306	0	0.00
	Total	144	22582	343	1.52*

* Mean

until December 1988. The filariasis situation in different panchayats, as determined by health camps, is presented in Table: 1.5.

1.9.5. Followup studies:

a. Effect of treatment on lymphodema:

A total of 56 recent oedema (Grade I lymphodema) and 70 persistent (Grade II lymphodema) cases was followed up longitudinally after treatment. Apart from Di-ethyl Carbamazine (each course 6mg/kg/day for 12 days), anti-inflammatory, antibiotics and crepe bandages were given as per necessity. The clinical course was monitored by water displacement test. Reduction in degree of lymphodema was observed in 38 (68%) recent and 32 (46%) persistent oedema cases.

b. Effect of DEC on microfilaraemia:

DEC was administered at the dosage of 6mg/kg of body weight per day for 12 days to 67 microfilaria carriers. Clearance of parasitaemia was observed in 59 (88%) of the cases following the single course treatment. A reduction in mean microfilaria count (pre 17.4, post 3.5) was observed in the remaining 8 persons. However after a second course of DEC these 8 persons also became amicrofilaraemic.

c. Filarialfever attacks:

To study the number of acute filarial fever episodes, 10, 81 and 38 cases of recent, persistent oedema and elephantiasis cases respectively were followed up for a duration of 6 months. All these patients were given a calender and asked to record the occurrence of filarial fever during the calender period. The average number of episodes prior to and after treatment was calculated from clinical history recorded in the calender. Reduction in fever episodes was observed in all groups and it was marked in elephantiasis cases.

1.10. Mass Drug Administration (MDA):

1.10.1. Annual single dose:

Mass DEC chemothreapy using annual single dose of 6mg/kg body wt. was carried out in Shertallai South Panchayat covering the entire population of about 33,000. Drug distribution was carried out through door to door visits, school camps (11 Nos.) and community camps (14 Nos.). A total of 18,265 persons were administered with DEC drug, the coverage being 66.07%. Side reactions following drug intake were observed among 3.5% of the population. Preliminary evaluation of annual single dose of DEC, in the panchayat was carried out by analysis of microfilaraemia in pre and post MDA conducted in the area. A total of 1752 persons were examined in pre MDA in 12 FDCs and 2255 persons in post MDA in 16 FDCs. The prevalence of infection was found to be reduced from 2.28% to 1.33%. The MFD 50, pre and post MDA, was 8.06 and 4.51 respectively.

1.10.2. Low dose DEC treatment given *BY THE PEOPLE FOR THE PEOPLE:*

Mass Drug Administration by VCRC through door to door visits not only requires greater efforts and inputs, but also leaves the community as passive recipients. In order to make the people realise the importance of chemotherapy and to actively involve them in the modified programme of action '*BY THE PEOPLE FOR THE PEOPLE*' was initiated. Towards this direction, community volunteers who are readily acceptable to the local public were identified through the member organizations of FILCO. After giving a thorough orientation, they were involved in DEC drug administration to the community. VCRC has taken the task of helping and guiding the workers apart from monitoring the programme.

i. Biannual single dose:

In Mararikulam North Panchayat, 75 community volunteers administered the drug to 5309 persons (93% of total population) with DEC biannual single dose. The usual 6mg/kg body wt. was given in three split doses in three consecutive days and thereby reduced possible side reactions. The volunteers were also provided with analgesic/antipyretic tablets to combat simple reactions such as headache and fever.

ii. Monthly single dose:

In one of the highly endemic villages (Chethy, Mararikulam North panchayat) a monthly single dose DEC administration (6mg/kg) was initiated for 600 people, from November 1988. A full course of the drug regimen is expected in a year

TABLE 1.5

Panchayat wise results of health camps.

Sl. No.	Name of Panchayat	No. of Camps	Clinical Screening		Parasitological Screening	
			No. Examined	mF Rate	No. Examined	mF Rate
1.	Shertallai South	2	904	15.38	873	5.15
2.	Kadakarapalli	1	360	23.61	355	0.85
3.	Shertallai Municipality	1	700	11.71	540	2.04
4.	Pattanakad	1	514	9.14	412	1.46
5.	Kuthiyathod	1	385	8.31	236	0.00
6.	Muhamma	1	538	2.23	514	0.39
7.	Thannirmukom	3	1323	4.84	1121	0.62
8.	Thykattussery	1	623	4.65	555	0.36
9.	Kanjikuzhi	1	458	3.49	514	0.39
10.	Pallipuram	1	567	1.94	387	0.00
11.	Thuravoor	1	1080	2.96	733	2.32
12.	Perumbalam	1	495	1.41	183	0.00
13.	Mararikulam North	1	507	4.54	501	5.79
	Total	16	8454	6.85*	6924	1.79*

* Mean

long period by this programme (12 × 6mg/kg). Fifteen active volunteers of a member organization (Venus Arts Club) of FILCO are engaged in this programme. This strategy is expected to boost the confidence and self-reliance of the community in health care, besides reducing the side effects encountered in standard dose regimen.

1.11. Health Education and Community Action Programmes:

1.11.1. Health Education Tools:

As a continuation of last year's *School Health Education Programme*, time table cards and name labels have been designed with new messages on disease vector control and distributed among school children of all Primary and Higher Secondary Schools of Shertallai. Measuring scales for school going children were also designed with attractive messages. Since a large population of Kerala commute regularly in State Road Transport Buses, messages on filariasis and its control are displayed in the transport buses plying from and to Shertallai. In order to cover wider area under health education campaign, new hoardings were erected at different vantage points in different parts of Shertallai taluk with attractive and meaningful messages.

In the District Science Fair held at Shertallai, one of the Students' Filariasis Control Clubs exhibited visual aids on various disease vector control measures. A stall on filariasis control was put up at the Agricultural Exhibition at Ponnittassery.

Various local folk arts continued to be a major component of VCRC's Community Health Education Programmes. These programmes were staged by talented FILCO volunteers. A popular dance 'Kayar Pinnal Kolkali' incorporating vector control as its major theme is being performed in all Health Camps by a troop of young volunteers belonging to the women's wing of FILCO. It has attracted wide public attention.

The VCRC has produced a documentary film (16mm) 'Yudham' (war) in Malayalam with a captivating story on the sufferings of filariasis victim. What the community could do in the control of this disease to which social stigma is attached is also well depicted. It is being screened in various parts of Shertallai. Many voluntary organizations are seeking the screening of this film for their functions which shows its popularity and significance.

The 'FILCO Movement' has grown into a major force with about 13000 active volunteers from 75 voluntary organizations in its roster. FILCO is engaged in varied activities to help VCRC in combating filariasis. One of the achievements of FILCO in collaboration with VCRC was to convert health education campaign from a vertical to a horizontal programme. The orientation classes aimed at creating an awareness on filariasis to the FILCO volunteers by VCRC has been extended to different corners of Shertallai region through a net work system formulated by FILCO.

'Shramadaans' (voluntary labour) for weed removal has become a regular feature of FILCO. Shramadaan philosophy has been extended to various parts of the taluk through the member organizations of FILCO. Filariasis Detection Camps to detect and eliminate the parasite load from the community is another major task being accomplished by FILCO. Sixty eight such camps were conducted during the reporting year. Through Health Camps and Filariasis Detection Camps, over 11000 people were parasitologically screened till December 1988.

1.11.2. Students' Organization for Filariasis Control:

The most vulnerable section of the community, the students, were given intensive health education to root out the misbelief and superstitions about filariasis and to create a future generation who are fully informed of all aspects of this disease. A sense of responsibility was inculcated among the students with regard to the problem of filariasis. Appropriate avenues to serve the community was made available to students with a view to involve them actively in the programme. Students' Filariasis Control Clubs formed last year have extended to 30 High Schools with 900 student volunteers representing the entire taluk. The main objective of forming these clubs is to involve the students as active participants in disease vector control activities in an organized

manner. One teacher from each school was entrusted with the responsibility of providing the necessary guidance for each club. The 30 schools having students clubs for filariasis control were divided into 4 zones with each zone covering 6–8 schools. The activities were carried out on a phased manner zone-wise. The goals envisaged for students' club are as follows:

i. Each club will be taking care of about 100 ponds around the vicinity of their respective schools for deweeding and fish culture.
ii. Organising of filariasis detection camps for the benefit of residents around the school premises.
iii. Mobilising the community to participate and utilise the services of VCRC.
iv. Organising 'Shramadaans' on holidays and during vacation.

At the initiative of Students' Clubs, over 1000 ponds have been deweeded in different locations and of these 887 ponds were brought under fish culture. Four Filariasis Detection Camps were also arranged by Students' club, through which over 900 people were parasitologically screened. An area of about 3350 sq.m. was made free from vector breeding by the student volunteers through 'Shramadaans'.

In order to encourage and to generate enthusiasm among the students on this subject, competitions were held on topics on filariasis and its control. The response from the student community was encouraging and the winners in the competition were given recognition by presenting prizes and trophies.

1.11.3. National Service Scheme:

National Service Scheme (NSS) of colleges are known for the services they render to community. Filariasis being the number one public health problem in Shertallai, the attention of the NSS of two colleges (NSS College and ST. Micheal's College) at Shertallai was directed towards filariasis control programmes.

The NSS students of St. Micheal's college devoted their entire Christmas vacation period of 10 days for disease vector control programme in one of the highly endemic villages, viz, Arthinkal with 'Shramadaan' covering over 3000 man hours. An area of about 35000 m^2 was made free of weeds and thereby preventing vector breeding. The students also engaged themselves in a vigorous health education campaign, as a result of which over 500 individuals were subjected to parasitological screening for filariasis during their vacation camp.

A ward in Vayalar Panchayat has been brought under the adoption scheme of a local College for filariasis control. A population of over 25000 is being protected from the risk of filarial infection by the students. Besides their efforts in the detection and elimination of prasite load in the community, their 'Shramadaans' in weed infested ponds made an area of 13,000 m^2 free from vector breeding. They also brought over 150 ponds under fish culture with the assistance of VCRC.

The activities are designed in such a way to make it 'SERVICE DURING LEISURE TIME' without interfering with their studies. The activities are, therefore, restricted to weekly holidays and vacations.

A 'Theruvu Natakam' (Street Drama) is being staged in street corners by the students of NSS of St. Micheal's College, and is attracting wide public attention in view of its health education and entertainment values.

1.11.4. Monitoring pre-school going children:

The network of Anganwadis functioning under the ICDS in this area, is expected to take care of pre-school children (one Anganwadi for 1000 population). Since the main objective of the Science and Technology Mission Project is to protect the future generation from filariasis, children below 6 years are receiving major attention of VCRC. So far, about 1200 children have been parasitologically screened in collaboration with Anganwadi workers. These children are being regularly examined and followed up.

DISEASE PREVALENCE:				: Range (%)
Disease Rate	...	...	...	: 11.6–26.1
mF Rate	...	...	...	: 1.2– 7.2
Endemicity Rate	...	...	...	: 12.8–33.3
HEALTH CARE DELIVERY: (Population benefited through medicare)				
Sample Survey	...	...	...	: 30442
Clinic at the Centre	...	...	...	: 26135
Filariasis Detection Camps (147 Nos.)		...	...	: 22963
General Health Camps (16 Nos.)	...	...	...	: 8454
Annual single dose DEC administration		...	...	: 18265
Biannual single dose DEC administration		...	...	: 5309
Monthly low dose DEC administration		...	...	510
HEALTH CARE IN SCHOOLS:				
No. schools visited	...	...	...	: 11
No. children given mass drug administration		...	...	: 3832
FISH CULTURE:				
No. ponds enumerated	...	...	...	: 75000
No. ponds deweeded	...	...	...	: 35837
No. ponds stocked with fish	...	...	...	: 35690
COMMUNITY PARTICIPATION:				
Health Education	...	...	...	No. Classes
Schools	...	...	...	: 127
Community	...	...	...	: 302
SHRAMADAAN:				
No. voluntary organisations involved		...	...	: 69
Area cleared	...	...	...	: 66250 M^2

2. MALARIA STUDIES IN KORAPUT DISTRICT OF ORISSA STATE:

Malaria has been persisting for several decades in the predominantly tribal populated Koraput District, Orissa State, India. Preliminary studies indicated that the epidemiology of malaria varies not only within different geoclimatic zones but also with topography within same geoclimatic zone. Hence six areas were delieated for carrying out in-depth studies on the reasons for the persistence of malaria and to develop an appropriate strategy for malaria control.

Mass Blood Surveys were conducted by VCRC in 61 villages, which included 12 tophill, 3 foothill and 19 plain villages in Borigumma Primary Health Center (PHC) area, 1 foothill village and two plain villages in Malkangiri PHC area and 24 top hill villages in the Bonda hills in the Khairput PHC area. The VCRC also made a one time sample survey at an irrigation project site at Muran, 65 Kms away from Jeypore frequented by migrant labourers and collected blood samples from a labour camp. The result of these surveys are summarized in Table 2.1 and fig 2.1.

Age specific analysis of prevalence indicated a high level of acquired immunity among the population. The lower prevalence and incidence among infants compared to young children is due to immunity conferred by mothers. This could also be due to lack of P-aminobenzoic acid in milk necessary for parasite growth, apart from presence of secretory antibodies which confer immunity. Since malaria is persistent for several years, young children between 2–5 years are the worst affected in this area.

The high prevalence of afebrile parasite carriers (85%) in the population indicates that the population had high acquired immunity. Asymptomatic parasitemia is known to be high in a immune population. In endemic areas where transmission continues through out the greater part of the year the population develops and maintains a high degree of immune response, while at the same time low parasitaemia persists in the population. Age specific spleen rates among children below 10 years of age also indicated age specific acquired immunity. One of the interesting aspects of *P. falciparum* infection in the locality is the paucity of gametocytes though a large proportion of the population had the infection. This could be due to innate immunity of the population, which has been exposed to infection for years.

The present study showed that the district is mesoendemic for malaria, though there were many pockets of hyperendemicity particularly in tophill and foot hill villages. The prevalence and incidence pattern of malaria varied in different geoclimatic zones.

2.1. Epidemiology of malaria in different geoclimatic zones:

2.1.1. Plasmodium ovale:

Detailed studies on malaria in Koraput district carried out since April 1988 showed that all the four species are prevalent in the district with predominance of *P. falciparum*. While all the four species are present in top and foot hill villages in Boriguma PHC area, *Plasmodium ovale* has not been recorded so far from other areas. This is the first report of *P. ovale* from India. Out of total of 748 blood smears collected (Table 2.2) nine cases were found positive for *P. ovale*. The oval shaped infected erythrocytes, heavy stippling in the early ring stage and fimbriated ragged process in erythrocytes which are characteristic of *P. ovale*. (plate 1) was observed in the blood smears collected from these patients. The identification of *P. ovale* was confirmed by Professor G.A.T. Targett and Prof. R.E. Sinden of the Imperial College of Science and Technology, London. The details of the positive cases are given below.

Case No. 1: A blood slide collected and examined on 18-7-1988 from a female named Hira, daughter of Hara Paiko, aged 14 years, from Champapadar village was found positive for both *P. ovale* and *P. falciparum*. At the time of slide collection the patient gave a history of fever for eight days. Fever was not accompanied by rigor or chill and the patient showed no other abnormalities. The patient was treated with 1500 mg of chloroquine to which patient responded positively.

TABLE 2.1

Result of malaria survey in different physiographic regions of Koraput district

Type of village	Top hill	Foot hill	Plain	Riverine	Foot hill	Top hill**
Locality	Borigumma	Borigumma	Borigumma	Borigumma	Malkangiri	Malkangiri
Altitude in metres (approximately)	900	600	600	600	150	900–1200
No. of villages	12	3	17	2	3	24
Total population	1645	2334	10769	2200	774	4370
No. examined	378	1608	6771	1309	667	1409
Coverage (% popln.)	22.98	68.89	62.9	59.5	86.18	32.24
No. + ve (%)	206 (54.5)	107 (6.6)	193 (2.9)	26 (1.99)	301 (45.1)	771(54.7)
No. Pf (% of all + ves)	192 (93.2)	95 (88.8)	177 (91.7)	22 (84.6)	228 (75.8)	568 (73.7)
No. Pv (% of all + ves)	5 (2.4)	11 (10.3)	16 (8.3)	4 (15.4)	50 (16.6)	82 (10.6)
No. Pm (% of all + ves)	9 (4.4)	0	0	0	3 (1.0)	40 (5.2)
No. mixed (% of all + ves)	0	1 (0.9)	0	0	20 (6.6)	81 (10.5)*
No. Gametocyte (% of all + ves)	19 (9.2)	15 (14.0)	18 (9.3)	1 (3.8)	74 (24.6)	145 (18.8)
Infant parasite rate (%)	73.33	0.00	1.2	0.00	50.0	60.00
Asymptomatic parasite carriers (% of all + ves)	125 (60.7)	90 (84.1)	80.1	22 (84.6)	255 (84.7)	674 (87.4)
Spleen rate (2–9 years)	63.75	7.03	2.5	0.00	53.06	52.9
A.E.S*	1.69	2.0	2.0	0.00	2.18	2.11

* Average Enlarged Spleen.
** Bonda Hills

TABLE 2.2

Malaria situation in top hill villages of Borigumma

Month	Number sampled	Number positive	Number *P. falciparum*	Number *P. vivax*	Number *P. malariae*	Number *P. ovale*	Number mixed
April '88	65	21	12 (1)	7 (2)	0 (1)	0 (0)	2
May	92	45	28 (5)	7 (4)	4 (2)	0 (0)	6
June	61	24	14 (0)	7 (0)	2 (0)	0 (0)	0
July	105	64	51 (6)	6 (5)	0 (1)	0 (2)	7
August	110	51	39 (5)	9 (5)	0 (1)	0 (1)	6
September	78	36	24 (3)	5 (4)	2 (1)	0 (0)	5
October	89	39	29 (7)	1 (7)	1 (0)	0 (1)	8
November	57	25	17 (2)	3 (2)	3 (0)	0 (0)	2
December	91	52	37 (10)	1(11)	2	1 (5)	11
TOTAL	748	357	251 (39)	46 (40)	14 (6)	1 (9)	47

No. of mixed infections shown in parenthesis

"Such moments (discovery of the role of mosquito in malaria transmission) comes only to one or two persons in a generation. The pleasure is greater than that given by any triumph of the orator, the statesman, or the conquerer; for the end attained does not lie in some petty intertribal advantage but in a benefit conferred upon all men and not only for today but for all time — atleast until "the future dares forget the past." The triumph of 20th August ~~1987~~ 1897 was now completed and crowned by that of 9th July 1898 — more than enough to compensate me for all my toils."

— SIR RONALD ROSS (1922).

in *"The Great Malaria Problem and its Solution"*.
From the memoirs of Ronald Ross.

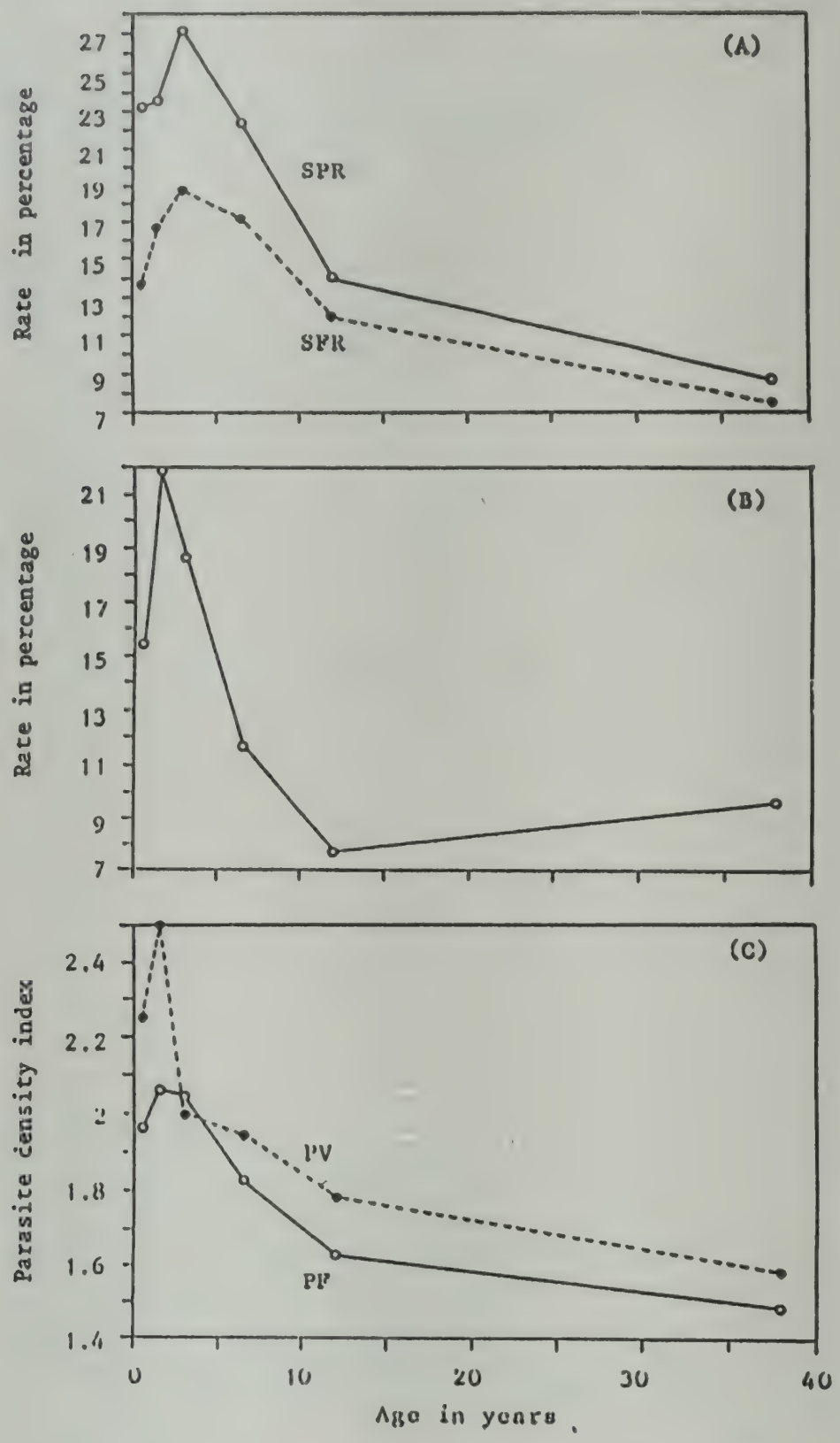


Figure 2.1 Agewise results of mass blood survey in 61 villages of Koraput (A) Slide Positivity Rate (SPR) and Slide Falciparum Rate (SFR), (B) *P. falciparum* gametocyte rate and (C) Parasite density index in *P. falciparum* (PF) and *P. vivax* (PV).

The patient never visited any other place outside the area.

Case No. 2: A female child, aged 4 years, named Subha, daughter of Danapathy Mudali, of Masipadar village was found positive for *P. falciparum* and *P. ovale*. Slide was collected and examined on 18-7-88. The patient had fever for four days which is accompanied by headache and body ache but rigor and chill was absent. The patient was treated with 375 mg of chloroquine to which the patient responded positively. The patient had never moved out of village since her birth.

Case No. 3: A 7 month old male infant, named Kuma, son of Pitambar Gowda of Masipadar village was found positive for *P. vivax* and *P. ovale*. Slide was collected and examined on 17-8-88. This patient was treated with 50 mg of chloroquine and was referred to nearby health center for radical treatment. The same patient was found positive again for *P. falciparum*, *P. vivax* and *P. ovale* on 5-10-88 and 5-12-88.

Case No. 4: A three month old male child named Surendra of Masipadar village was found positive for *P. ovale* and *P. vivax* infection on 5-12-88. Spleen was not palpable. The patient was treated with 50 mg of chloroquine and was referred to primary health center for radical treatment, Neither the parents nor the child had moved outside the village.

Case No. 5: A six and half year old female child named Domai from Masipadar village was found positive for *P. ovale*, *P. falciparum* and *P. vivax* on 5-12-1988. At the time of blood smear collection the patient gave history of intermittent fever since 15 days. The patient was treated with only chloroquine to which patient responded. Spleen examination showed grade I condition. Patient also did not give any history of movement outside the village.

Case No. 6: A three year male child named Yudhistir suffering from continuous fever was found positive for *P. ovale* along with *P. falciparum* and *P. vivax* on 5-12-88. Spleen was not palpable in this case. Epidemiological investigation indicate that the case is indigeneous from Masipadar village.

Case No. 7: A 9 month old male child named Prasanto from Masipadar village was suffering from continuous fever and was found positive for *P. ovale* and *P. vivax* on 22-12-88. Spleen was normal and not palpable. This case was also indigenous.

Case No. 8: A male child of one and half years from Champapadar village namely Bhagwan Paikc was found positive for *P. ovale*, *P. falciparum* and *P. vivax* on 5-12-88. This was also a indigenous case with grade IV spleen.

Case No. 9: A two year old female child from Champapadar village namely Domai daughter of Kamallochan Paiko was found positive for *P. ovale* and *P. vivax*. At the time of blood collection the patient was suffering from intermittent fever for past eight days. Spleen was not palpable and the case investigation shows that the case is indigenous.

Since man is the only known reservoir host of *P. ovale*, and there was no chance of introduction of the parasite from outside, it is clear that *P. ovale* has always been present in the area and due to uninvestigated reasons, had remained undetected till now. Champapadar and Masipadar are 3 kms apart, situated on the top of the hill ranges (altitude 800 m) and the population is tribal and had never been outside the district.

Presence of *P. ovale* from India, where malaria eradication program is in operation since 30 years is an epidemiological mystery since the parasite is known to be highly susceptible to antimalarials. The chances of introduction from other area is nil since inward and outward migration is absent due to remoteness of the area. The entire hilly tract of Koraput district is known to be hyperendemic for malaria with the dominance of *P. falciparum*. Though *P. falciparum*, *P. vivax* and *P. malariae* were recorded, no case of *P. ovale* was ever reported from any part of India. Earlier survey carried out in this region was by Perry in 1914 whereas characteristics of *P. ovale* was described only during 1922. Therefore it is possible if this species was missed by Perry. Subsequent survey carried out by Senior White 1940 also did not record *P. ovale* because both the surveys did not include Boriguma PHC area. Thereafter these areas were under the surveillance of National

Malaria Eradication Program (NMEP). However, the poor surveillance (or, almost the lack of it) in this area is responsible for missing the foci. Further, the majority of malaria cases detected in this area were mostly from the passive cases. It is worth mentioning that the information regarding severe cases and deaths due to malaria never reach the PHC in time, which is 60 km away. Since *P. ovale* produce only mild discomfort in the infected person, such cases might not have come to PHC. Quality of staining and examination in the PHCs is so poor that there is all the more possibility of missing *P. ovale*. Even a relatively common species like *P. malariae* is also missed and hence not reported by NMEP. The finding that the transmission of all the four human *Plasmodia* occur in this area is of great epidemiological interest from the point of view of malaria control and understanding of the disease process.

2.1.2. Seasonal incidence:

Fortnightly fever surveillance was carried out in top hill villages of Borigumma from April 1988 onwards and which showed that the transmission continues to occur throughout the period surveyed, April to December, with one sharp peak during July (Fig 2.2). The study is continuing and it would not be surprising if transmission occurs in all months. It was observed that fever due to causes other than malaria is high during August to Nov. To study the asymptomatic carriers and their role in malaria transmission mass blood surveys were carried out every month and the results are depicted in fig. 2.3. The results of mass blood survey which includes fever as well as non fever cases show that there are two peaks, one during May and another during July. Since a majority of the cases are asymptomatic, the second peak of prevalence is usually undetectable in fever surveys. The peak prevalence during May is mainly due to accumulation of older cases which do not have clinical symptoms, probably due to short term immunity built up in the population after the peak transmission period during monsoon (July). Infant parasite rate which is the real indicator of the transmission confirms that intense transmission takes place only during monsoon period.

Seasonal prevalence of malaria in the foot hill, plain and reverine villages followed a similar pattern except that the magnitude was lower in comparison to top hill villages (Fig 2.4–2.9). Moreover the peak of asymptomatic parasitaemia observed in top hill villages during May was absent in these groups of villages. This is probably due to the fact that the surveillance and chemotherapy is better in foot hill, plain and riverine villages due to easy access to these villages in comparison to top hill villages. Moreover, whereas the VCRC started surveillance and chemotherapy in foot hill, plain and riverine villages from January 1987, studies in top hill villages were initiated only from April, 1988.

The study shows that the prevalence of malaria is highest in top hill, followed by foothill, riverine and plain villages. The analysis of data from the Malkangiri Plateau showed that the pattern of prevalence (Table 2.1) and incidence (Fig. 2.10–2.13) was similar in top hill villages (Bonda Hill). However, in foothill and plain village of Malkangiri plateau one additional peak in March was observed which was absent in Jeypore plateau. This is mainly due to the fact that the irrigation facilities provided in Malkangiri area by the Dandakaranya project provides enough breeding grounds for the vector species in summer months. In foot hill villages of Malkangiri the second peak is shifted to October in comparison to Jeypore plateau. While there was a sharp monsoon peak in July in Jeypore area, in Malkangiri there was a gradual rise from April and the peak was observed during October (Fig: 2.11). In the plain villages of Malkangiri plateau the prevalence is lower than in foot hill villages and the second peak is seen during November. In both groups of villages in Malkangiri it was observed that fever cases other than those due to malaria are very high throughout the year. This difference in malaria prevalence in the two plateaus is mainly due to difference in climatological conditions. In Malkangiri area the temperature is more conducive for rapid completiton of sporogony cycle.

2.1.3. Vector prevalence:

All the 22 species reported earlier are present in all villages though there is a wide variation in numbers between the villages. While *A. fluviatilis* and *A. jeyporiensis* are very common in top hill and foot hill village, *A. annularis* and *A. culicifacies* are more common in plain and riverine villages. Among the vector as well as non vector species,

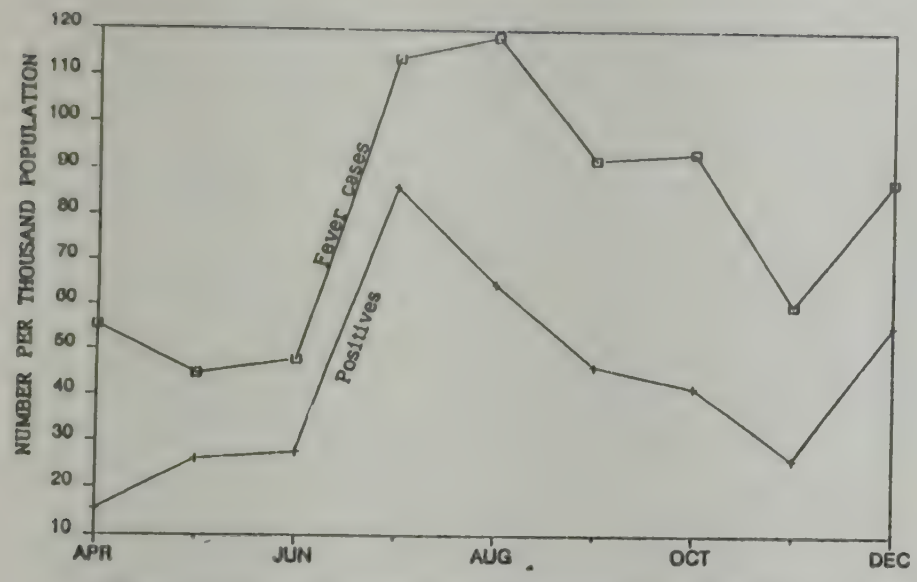


Figure 2.2 Fever Survey. Top hill villages–Borigumma

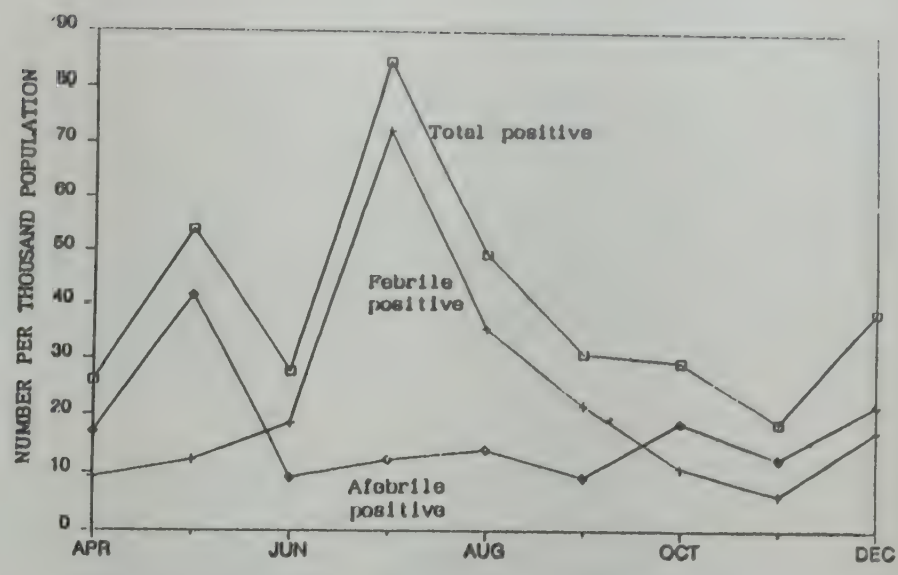


Figure 2.3 Mass Blood Survey. Top hill villages–Borigumma.

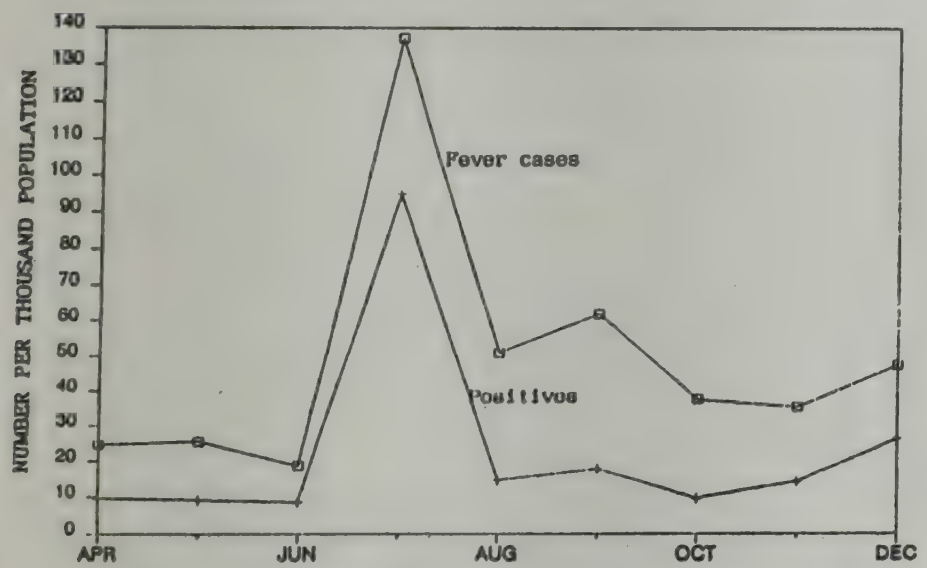


Figure 2.4 Fever Survey–Foot hill villages–Borigumma.

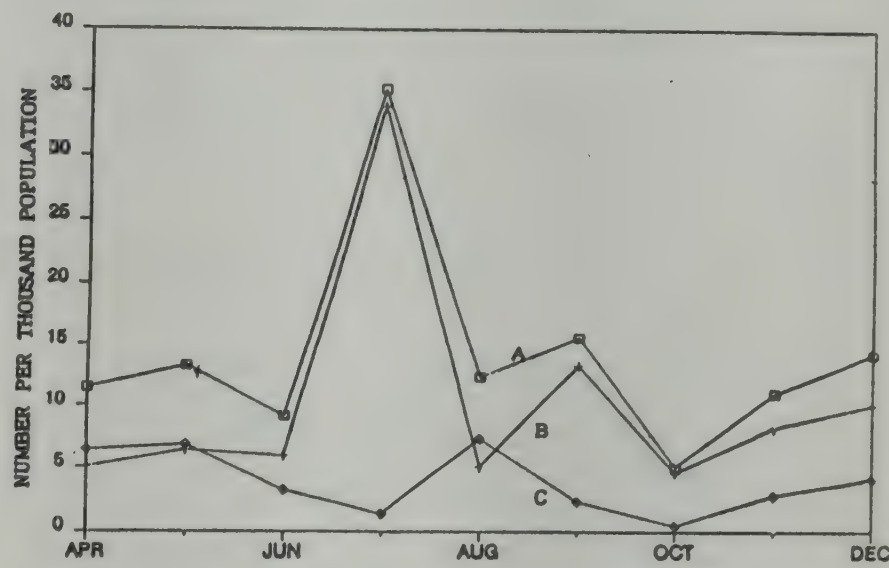


Figure 2.5 Mass Blood Survey–Foothill villages–Borigumma
A–Total positives, B–Febrile positives,
C–Afebrile positives.

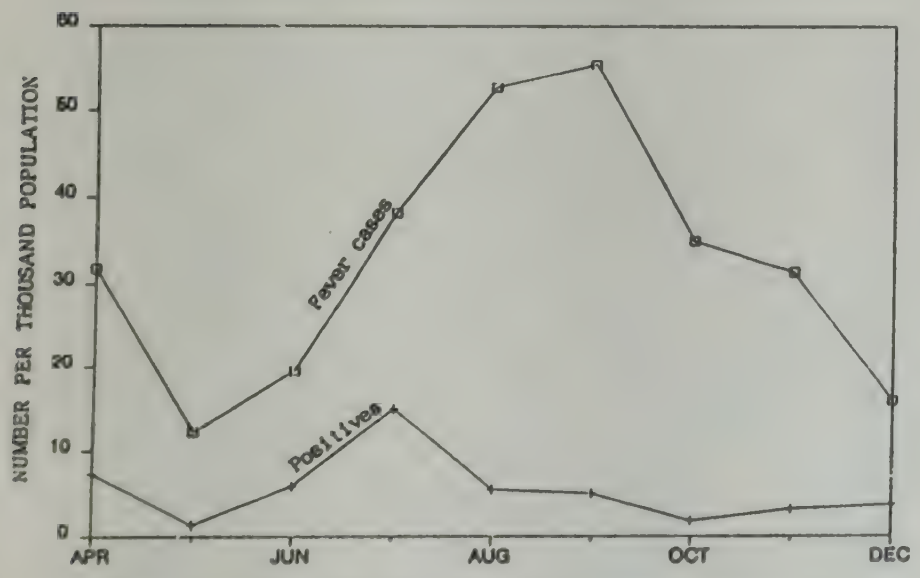


Figure 2.6 Fever Survey- Riverine villages–Borigumma.

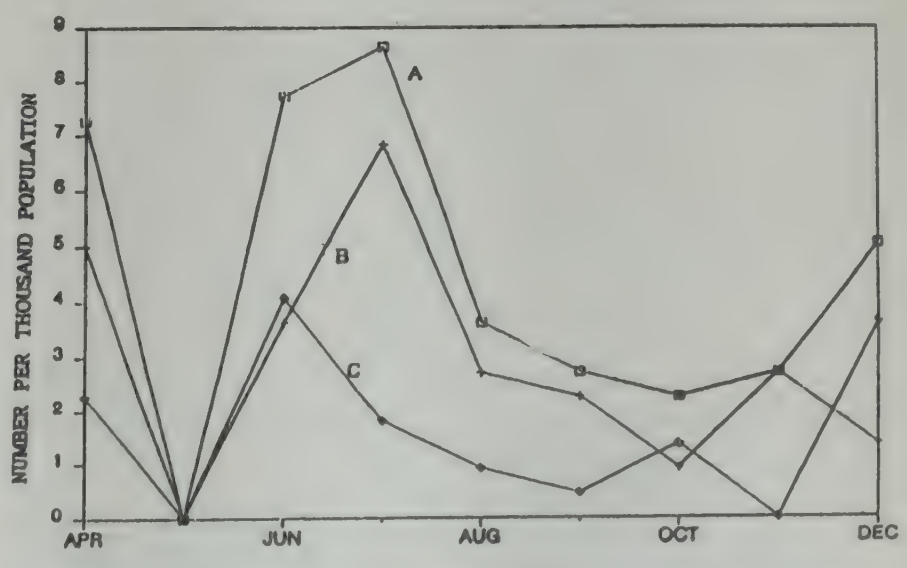


Figure 2.7 Mass Blood Survey–Riverine villages–Borigumma
A–Total positives, B Febrile positives,
C–Afebrile positives.

5A

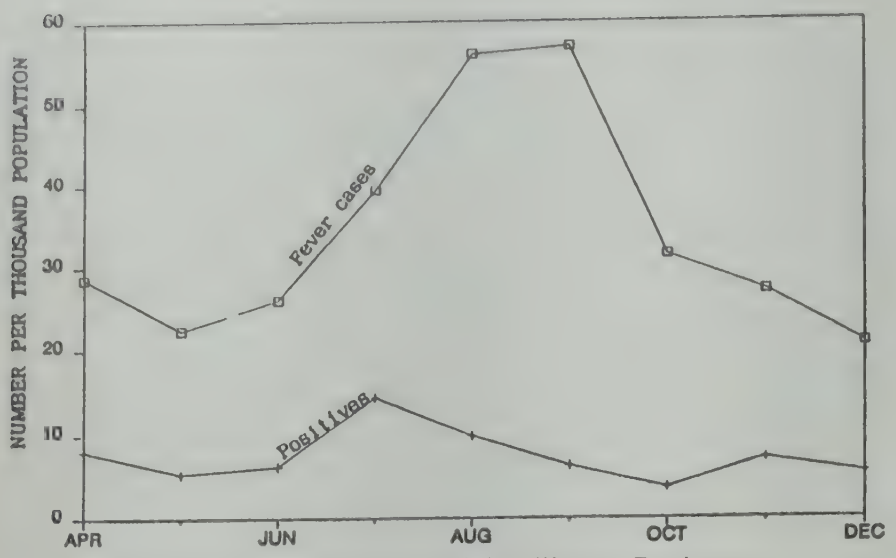


Figure 2.8 Fever Survey–Plain villages–Boriguma

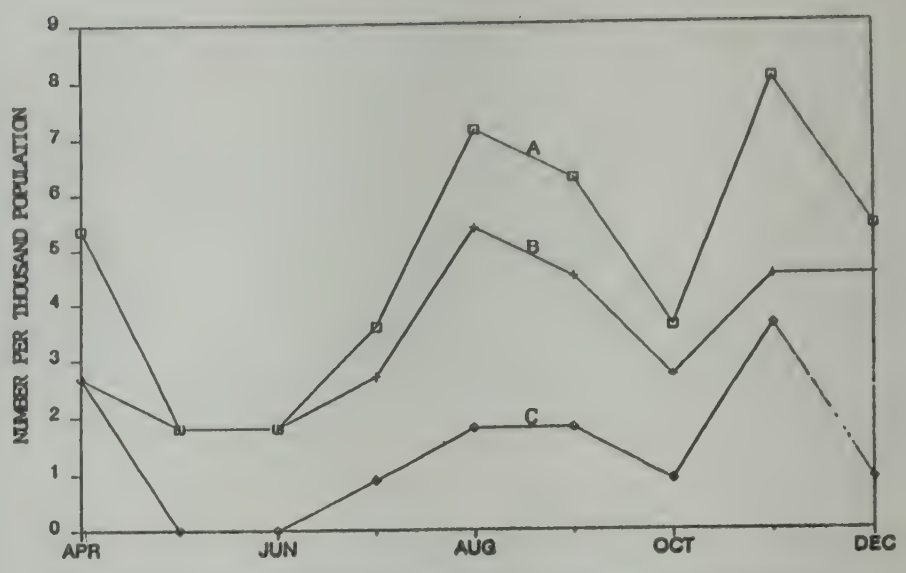


Figure 2.9 Mass Blood Survey–Plain villages–Borigumma.
A–Total positives, B–Febrile positives,
C–Afebrile positives.

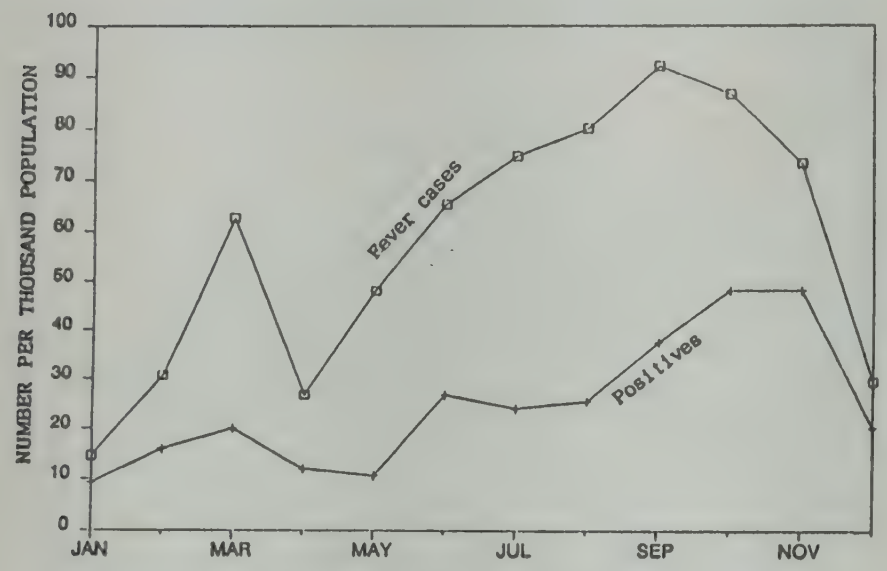


Figure 2.10 Fever Survey–Foot hill villages–Malkangiri

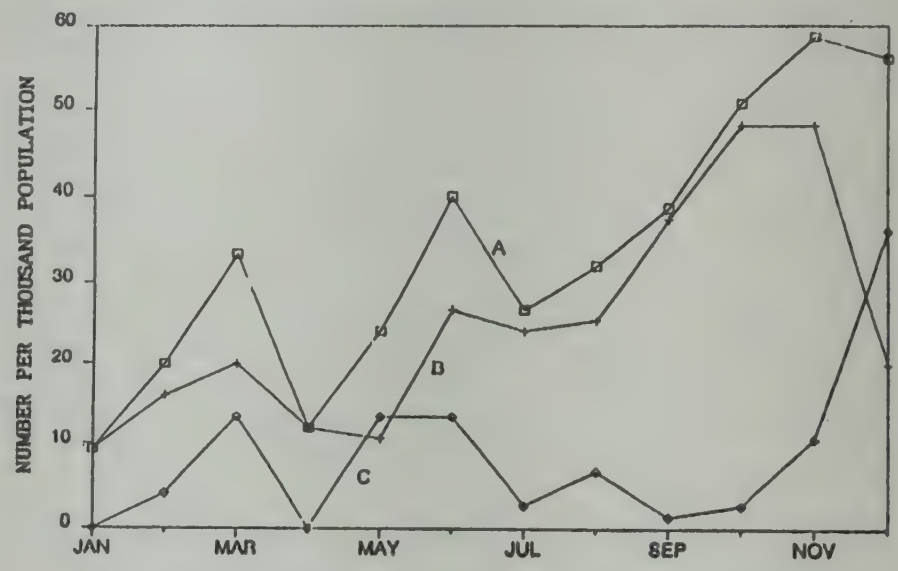


Figure 2.11 Mass Blood Survey–Foothill villages–Malkangiri
A–Total positives, B–Febrile positives,
C–Afebrile positives.

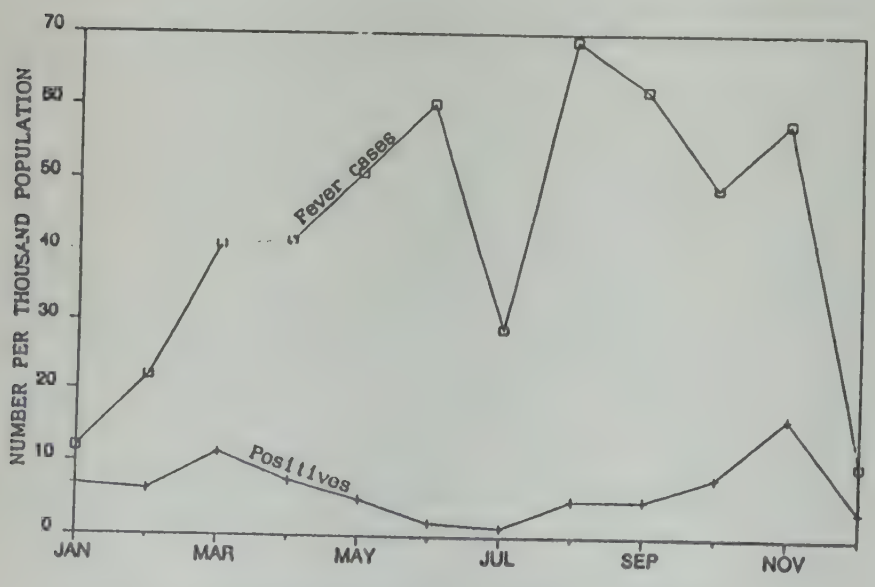


Figure 2.12 Fever Survey–Plain villages–Malkangiri

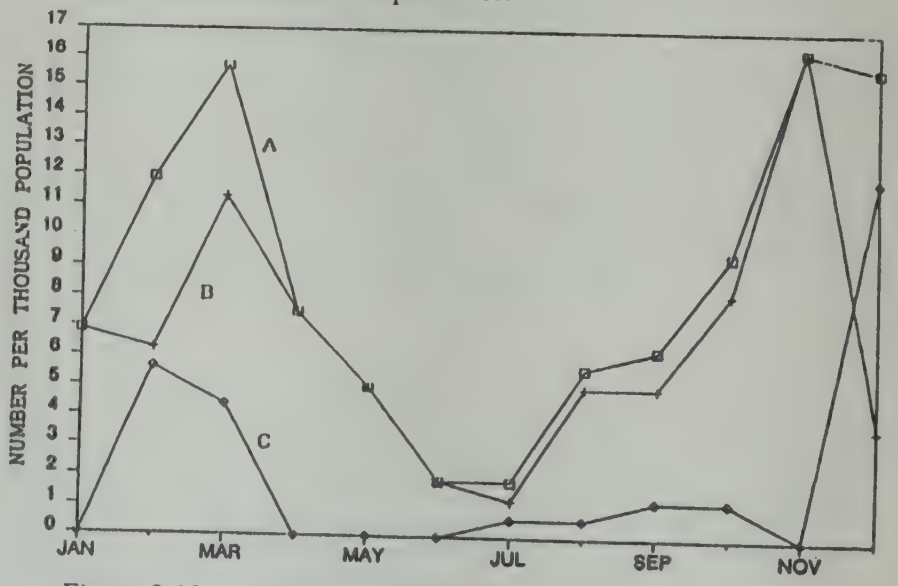


Figure 2.13 Mass Blood Survey–Plain villages–Malkangiri
A–Total positives, B Febrile positives
C–Afebrile positives.

A. culicifacies is most abundant. The relative abundance of different species is shown in table 2.3. Analysis of seasonal prevalence of different vectors showed that while *A. culicifacies* and *A. annularis* were present throughout the year, prevalence of other vector species was seasonal.

2.1.4. Vector incrimination:

A total of 4402 female anopheline mosquitoes was dissected for presence of parasite. In Borigumma, sporozoite was detected in one *An. fluviatilis* (September, 1987) and one *An. annularis* (October, 1988). Oocysts were detected in *An. fluviatialis*, *An. culicifacies* and *An. annularis*. In Malkangiri area sporozoites and oocysts were detected in 5 *An. fluviatilis* during December, 1988 alone. While the Vectorial role of *An. fluviatilis* and *An. annularis* is confirmed, the vectorial status of *A. culicifacies* has been confirmed only by oocyst positives. Intensive efforts are continuing to evaluate the role of other anopheline vectors in the area.

Since the relationship between the density and infection in vector and malaria transmission in the population is not clear, it is felt that the collection methods are not sensitive enough to give a clear picture. Hence different methods were used for collection and it was found that light traps are efficient in collecting *An. fluviatilis* and *An. annularis* (table 2.3). Even the total catch by indoor pyrethrum spray was not as productive as light trap.

2.2. Malaria among different tribal communities:

The analysis of data on malaria prevalence in different communities is presented in tables 2.4 to 2.6. The prevalence of infection was high in aborigine communities of Kondhs, Bondas and Porojas. Prevalence was higher in children compared to adults in all communities excluding the Omnathios. The tribewise comparison showed that the spleen rates are high among Kondh, Bonda and Poroja children. Statistical analysis showed that there is a significant correlation between spleen and parasite rate ($r = 0.99$ $P = < 0.0001$). The annual incidence also varied among different communities but the pattern was not similar to that observed for prevalence. Annual parasite incidence was highest among the Omnathios and lowest among Porojas. Prevalence was very high among Bondas and Kondhs, but these two tribals live in isolated top hill villages, where the climatological and ecological factors are more conducive for perennial transmission and no malaria control measures reach these tribals. Hence it is difficult to ascertain to what extent genetic factors influence malaria persistence. Genetic factors may have a role to play in the difference in malaria pattern among different tribals and need further investigation.

The role of genetic markers like ABO blood group, glucose 6 phosphate dehydrogenase (G6PD) deficiency and abnormal heamoglobin (Hb S) as in sickle cell anaemia are known to affect pattern of *P. falciparum* malaria. It is possible that these genetic factors may have some bearing in the disease distribution pattern, but other environmental factors conducive for vector breeding and availability of control measures are also being studied.

2.3. Chloroquine Sensitivity status of *P. falciparum:*

P. falciparum is the predominant parasite species accounting for about 90% of the infections in the area. Chloroquine is supposed to be administered by the NMEP to the *P. falcipaurm* cases in three different dosage schedules as described below.

i. A dosage of 600 mg chloroquine base is given as presumptive treatment at the time of blood smear collection. The positive cases were given an additional 600 mg chloroquine with 45 mg primaquine (adult cases) for radical cure.

ii. In inaccessible areas, a single dose of 600 mg base of chloroquine and 45 mg of primaquine is being given presumptively at the time of blood smear collection.

iii. Some cases of Schedule (i) receive only 600 mg chloroquine base given presumptively, as the radical treatment is not available due to logistic problems (non availability of drug/patient/staff etc.).

A total of 70, 43 and 35 *P. falciparum* patients were treated with dosage schedules i, ii and iii respectively by VCRC. They were followed for

TABLE 2.3

Anopheline species collected indoors, outdoors and in traps from Borigumma PHC villages (females only).

Species	Outdoors (Apr.–Dec.)	Indoors (Apr.–Dec.)	Traps (Aug.–Dec.)	Total
An. aconitus	4	123	457	584
An. annularis	9	154	731	894
An. barbirostris	0	65	798	863
An. culicifacies	51	5120	51	5222
An. fluviatilis	41	90	475	606
An. hyrcanus	0	13	1	14
An. jamesi	1	3	120	124
An. jeyporiensis	20	356	2113	2489
An. jeyporiensis var. candidiensis	0	25	1	26
An. karwari	0	0	20	20
An. maculatus	5	5	55	65
An. pallidus	3	68	1734	1805
An. philippinensis	0	9	11	20
An. ramsayi	0	0	23	23
An. splendidus	14	48	843	905
An. subpictus	13	2629	207	2849
An. theobaldi	0	2	15	17
An. tessellatus	45	9	114	168
An. vagus	55	5107	270	5432
An. varuna	5	48	126	179
An. nigerrimus	0	77	2326	2403
	266	13951	10491	24708

TABLE 2.4

Tribe wise parasite rate, gametocyte rate and density

Tribe/Community	Sample		Parasite Rate (%)		Afebrile P.R. (%)		P. falciparum Gam. Rate (%)		P. falciparum Density Index	
Age in Years	⟨ 15	⟩ 15	⟨ 15	⟩ 15	⟨ 15	⟩ 15	⟨ 15	⟩ 15	⟨ 15	⟩ 15
Kondh	59	107	71.1	38.3	69.1	85.4	9.9	10.5	1.61	1.57
Bonda	798	611	71.4	32.9	88.2	95.7	15.6	9.3	1.90	1.49
Porja	969	1738	8.0	5.2	63.6	75.8	5.5	10.8	2.08	1.60
Dombo	475	915	3.6	3.2	88.2	100.0	7.1	4.0	1.14	1.28
Omnathio	214	412	2.8	5.1	100.0	71.4	0.0	19.1	1.00	1.38
Paiko	47	123	4.3	1.6	100.0	50.0	0.0	0.0	2.50	2.50
Tanti	106	165	5.7	4.2	100.0	100.0	0.0	25.0	1.33	1.29
Mali	107	224	0.9	1.8	100.0	100.0	0.0	25.0	1.00	1.25

(P.R.:- Parasite Rate, Gam:- Gametocyte).

TABLE 2.5

Tribe wise spleen rate among children (2–9 years old)

Tribe/ Community	Sample size	Spleen Grade 1	2	3	4	5	Spleen Rate (%)	AES*	Parasite Rate (%)
Kondh	25	3	12	1	0	0	64.0	1.88	84.00
Bonda	537	40	184	50	9	1	52.8	2.10	76.50
Porja	122	10	10	2	0	0	18.0	1.64	19.70
Dombo	101	0	3	1	0	0	4.0	2.25	2.40
Omnathio	185	0	2	0	0	0	1.1	2.00	2.16
Paika	25	1	1	0	0	0	8.0	1.50	4.00
Tanti	29	1	2	0	0	0	10.3	1.66	3.44
Mali	47	0	0	0	0	0	0.0	0.00	0.00
Bhumia	57	1	1	0	0	0	3.5	1.50	0.00

TABLE 2.6

Incidence of fever and malaria among different tribes

Tribe/ Community	Population		Fever Incidence		Parasite Incidence		*P. falciparum* Gam. Rate (%)	
Age in Years	⟨ 15	⟩ 15	⟨ 15	⟩ 15	⟨ 15	⟩ 15	⟨ 15	⟩ 15
Porja	1491	2847	102.0	131.7	34.2	43.2	5.1	6.4
Dombo	920	1744	130.4	182.9	37.0	51.6	7.1	10.5
Omnathio	391	678	496.2	697.6	104.9	182.9	0.0	3.1
Paika	101	248	386.1	467.7	138.6	197.6	8.3	2.6
Tanti	116	210	336.2	261.9	94.8	52.4	0.0	9.1
Mali	179	312	217.9	333.3	50.3	80.1	0.0	15.0

(Incidence Estimated per 1,000 Population).

"The solution of the malaria problem has been called the most dramatic episode in the history of medicine, and the fact when disclosed were certainly among the most wonderful in natural history; but the story has often been very inaccurately reported.

. . . . . it is hoped that the work will be of some practical use as regards the reduction of one of the most widespread of diseases. Now this is a matter which is always ultimately in the hands of laymen — it is they, not the doctors, who rule the world. Hitherto the matter has been left almost entirely to the medical profession, which however has failed to carry my scheme of 1899 to its logical conclusion, largely because it is allowed little influence in the world's counsels. Nothing, I am convinced, will really be done in this direction until those who govern us take the trouble to understand the subject."

— SIR RONALD ROSS (1922)

in *"The Great Malaria Problem and its Solution"*.
From the memoirs of Ronald Ross.

persistence of parasitaemia on the 7th day of their receiving the drug.

The results showed the presence of asexual parasites in 18.6% (13/70), 9.3% (4/43), 34.3% (12/35) cases using dosage schedules i, ii and iii respectively on the 7th day following receipt of drug/drugs indicating possibility of prevalence of drug resistant strains in the locality. This indicates the reduced efficacy of chloroquine in the same dosage schedule over the years. Therefore chloroquine sensitivity of *P. falciparum* was carried out in 4 Primary Health Centre (PHC) and Muran project areas in the district.

In the *in-vivo* extended test 149 persons were included, of whom 139 (93.3%) were followed up upto 28 days, of whom resistance was detected in 11 at R_I and 3 at R_{II} level.

In-vivo standard test was carried out in Muran Project area and 16 out of 20 persons included were followed upto 7 days. The case selection and test were carried out as per WHO procedure. One case out of 16 showed resistance at R_{II} level.

Micro *in-vitro* chloroquine sensitivity test was done by using the WHO kit following standard procedure for 17 cases (11 from Borigumma PHC area and 6 from Muran Project area). Of these 8 were found to be resistant as they showed growth of schizonts at above 5.7 picamol chloroquine. All the six cases from Muran project area were found to be resistant.

Epidemiological investigation revealed that all the resistant cases were indigenous. Majority of cases were from Malkangiri and Muran. All the resistant cases detected by both *in-vivo* and *in-vitro* test, were treated with 1000 mg sulphamethopyrazine and 50 mg pyrimethamine (Metakalfin, Walter Bushnell).

The resistance of *P. falciparum* to chloroquine in the locality could be due to introduction of a resistant strain from outside or due to natural selection. There is a large refugee settlement (over 500,000 population) in Malkangiri area and these people who are originally from East Bengal (present Bangladesh), make frequent visits to Eastern part of the country (Bengal, Assam etc.), where resistant strains are known to exist. In Muran Project area there is a large congregation of local labourers and migrant labourers come from other States like Bihar, Kerala, Tamil Nadu, Andhra Pradesh, Maharastra, and Madhya Pradesh. The fact that a majority of the resistant cases were from these two areas indicate probable introduction of resistant *P. falciparum* strain from outside. However, the possibility of establishment of the resistant strain due to natural selection cannot be ruled out due to the following reasons. The parasite in these areas have been subjected to chloroquine pressure since a long time and the drug is easily available to people. The spread of resistant strain to other receptive areas within the district and outside is of great concern considering the fact that there is substantial degree of human movement for developmental purposes. Role of human movement in the spread and persistence of malaria cannot be over emphasized. Chloroquine should be administered in the dosage recommended for radical cure (1500 mg base for adults).

Metakalfin (sulfalene and pyrimithamine) has been recommended as the alternate drug in treating resistance cases in this area (according to PHC medical officers), which was employed in present study also. But this drug must be employed judiciously as resistance to this drug has already been reported in some parts of India. It is essential to establish monitoring centres at focal points to prevent spread of malaria including drug resistant strains...

3. ASPECTS ON PARASITE EPIDEMIOLOGY OF BANCROFTIAN FILARIASIS

The studies on Bancroftian filariasis was directed towards real situation analysis of epidemiological data set, so that appropriate model could be developed for epidemiological application, and experimental studies to test the validity of findings.

3.1. Analysis of Existing Data set:

Detailed analysis of the epidemiological and entomological data was done to (1) understand the parasite epidemiology in human and vector population, (2) Study the relationship of entomological and epidemiological parameters, and (3) examine the role of various factors including acquired immunity and density dependent factors in filarial infections. The parasitological and entomological data collected from 1981 to 1986 during the operation of the Filariasis Control Demonstration Project were used as the data base.

3.1.1. Distribution of parasite numbers per host.

To understand the transmission dynamics in filariasis, it is necessary to study the distribution pattern of parasite in host population. Though it is ideal to look into the distribution of adult parasite population in human host, it is not possible to enumerate the adults with the available techniques. Hence, the distribution of microfilaria (mF) in blood is studied which is an indirect measure of adult density.

The frequency distribution of microfilaria count in a population provides information about the biology of the host and the parasites concerned. Analysis of frequency distribution of mF counts showed that the negative binomial distribution was found to be a good fit for all age classes but for the age class 55–59 (Table 3.1). However we found that for mF counts of 0 and 1 the expected frequencies deviated much from the observed. Though the distribution of parasite is contagious, the chi square values reveal that the negative binomial distribution is not adequately describing the data, which is contrary to the expectation. Therefore the good fit may be due to the large degrees of freedom obtained from the large sample size. The reason for this discrepency is that the observed data show a considerable excess of zero counts compared with the negative binomial expectations. The problem is illustrated in Fig. 3.1 which shows observed and expected non-zero frequencies for the 15–19 year age group. The expected non-zero frequencies from a standard (non-truncated) negative binomial distribution show a much larger frequency at a count of 1 mF than the observed distribution. This is because the larger number of individuals with zero counts in the observed distribution which in turn generated a much more skewed distribution ($K = 0.03$) than is consistent with non-zero counts.

Since the parameter 'K' of this distribution is an inverse measure of aggregation, on both empirical and theoretical grounds, the negative binomial distribution should describe the observed pattern. Recently work carried out elsewhere has shown that the negative binomial distribution appropriately describes microfilaria counts in human host. The reasoning here is that microfilaria are distributed continuously (in a gamma distribution) in the blood and that the effects of blood sampling which is a Poisson process, together generate a negative binomial distribution for the overall counts.

However a truncated negative binomial distribution was found to be good fit of the observed microfilaria frequency distribution ($X^2 = 20.25$, $P > 0.05$). The aggregation parameter was also found to be higher ($K = 0.25$) compared to non-truncated distribution ($K = 0.03$).

The reason for a very high number of negatives (zeros) observed contrary to the expectation of negative binomial distribution is not exactly known. This may be due to lack of sensitivity of the sampling technique (20 mm^3 blood smear) to detect low level microfilaria in blood. If however, the observed frequency is real, more refined mathematical and statistical techniques need to be evolved to study the distribution and its biological implications.

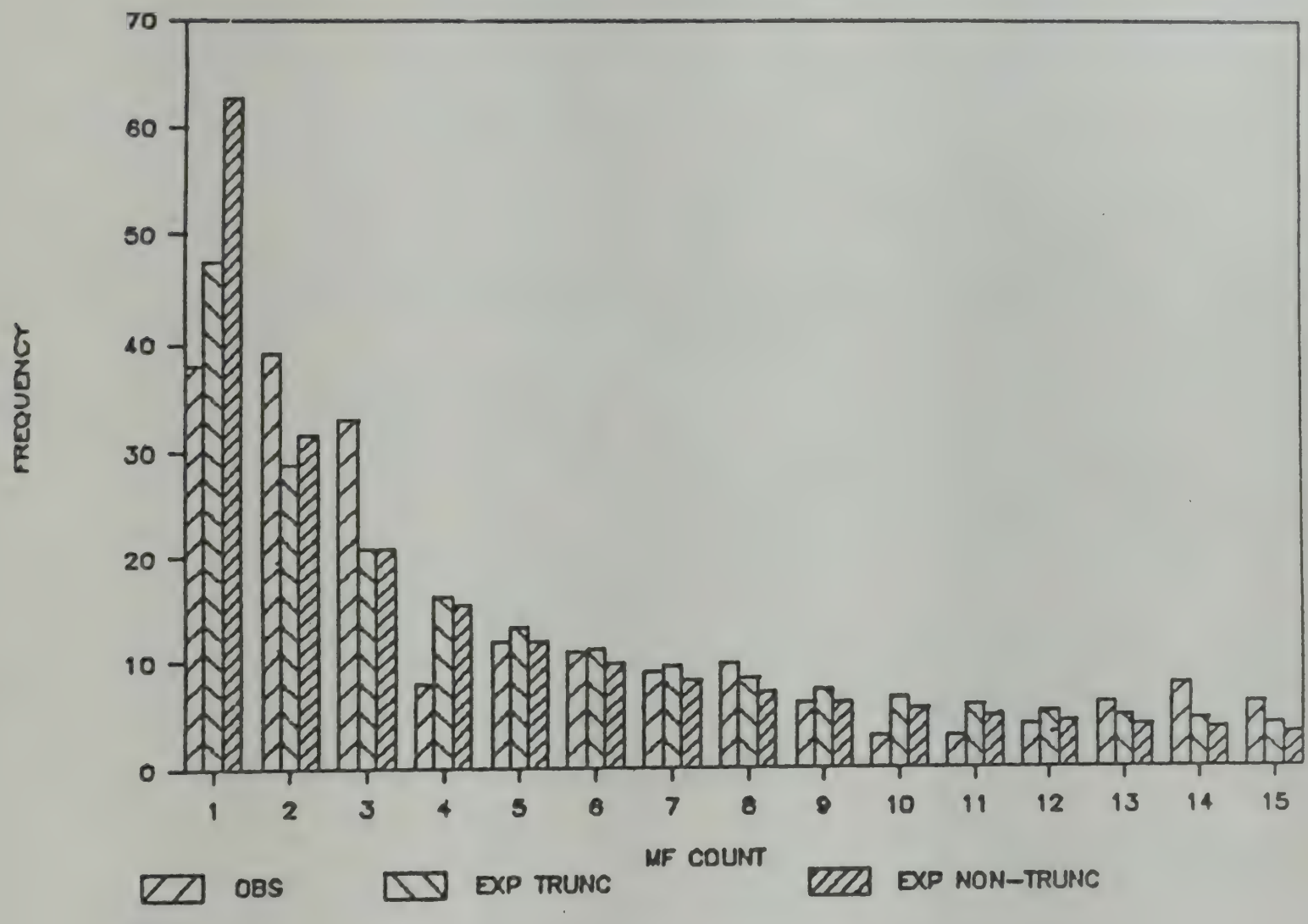


Figure 3.1 MF count negative binomial fits–For VCRC 1981; 15–19 Age group.

TABLE 3.1

Age wise negative binomial fitting for frequency distribution of MF counts in Pondicherry (1981).

AGE (Years)	K	Chi sq.	Df	P value
0–5	0.0068	48.7	41	0.19
6–8	0.0098	99.1	87	0.18
9–11	0.0206	80.4	90	0.76
12–14	0.0273	109.0	106	0.40
15–19	0.0308	110.9	99	0.19
20–24	0.0312	125.9	114	0.21
25–29	0.0305	110.9	152	0.99
30–34	0.0284	96.6	83	0.15
35–39	0.0265	53.1	58	0.66
40–44	0.0195	286.4	278	0.35
45–49	0.0249	69.7	55	0.09
50–54	0.0262	99.9	89	0.22
55–59	0.0212	86.5	57	0.01
60–64	0.0227	53.2	49	0.32
65–69	0.0240	51.8	50	0.41
>=70	0.0181	27.0	63	1.00

3.1.2. Loss and Gain of infection:

In the cohort, out of 631 persons who were mF positive in 1981, 390 had become amicrofilaraemic accounting for 62% loss of infection. Of the 6,894 who were amicrofilaremic in pre control survey 246 had become microfilaraemic accounting for 3.6% gain of infection. The age specific gain and loss in the cohort is shown in the Fig. 3.2. This indicated that the gain of infection ranged between 3% and 4.6% in different age classes and there was a significant ($X^2 = 4.8$; $P = 0.03$) rise from 3% in less than 10 year old to 4.6% in 11–20 years age class. It was more or less stable above 20 years of age. This could be due to higher rate of acquisition than loss in younger children as compared to adults in whom, the rate of gain and loss is balanced. Epidemiological analysis of the data shows that both intensity and prevalence of infection, also showed a similar trend as that of age specific gain of infection.

The loss of infection ranged between 56% and 65% and there was no significant difference between the age classes. This could mean that the loss of infection is purely a function of parasite fecundicity and survival and it is not influenced by any factor related to host age. Sex wise comparison of loss and gain did not reveal any significant difference.

The above estimation was done without taking into account the loss of infection due to chemotherapy in 1981. Out of the 631 mF carriers detected in 1981 and followed up in 1986, definite treatment history could be obtained only from 240 persons. A total of 171 persons did not receive any treatment and 69 persons received only one course of DEC (in the dosage of 6 mg/kg/day × 21 days). The difference in intensity of infection after a period of 5 years among the 69 who received treatment was significant ($t = 2.57$; $P<0.01$) where as it is not significant ($t = 1.34$; $P = 0.18$) in the 171 persons who did not receive any treatment.

The age specific analysis of natural loss and that due to chemotherapy ,showed that both curves are qualitatively similar but quantitatively different (Fig. 3.2). The loss due to therapy was uniformly higher compared to natural loss which was as expected. The loss was higher in young children less than 11 years of age, following which it decreased sharply until 15 years of age to rise and stablize in adult age classes. This could be due to low worm burden in children. The age intensity profile in both the surveys, had shown that intensity of infection is low in younger classes to rise and stabilize in adults.

3.1.3. Estimation of fecundic life span of adult female *W bancrofti*

Estimation of life span of *W. bancrofti* parasite is essential, as it will help to decide the duration of control measures and to understand the transmission dynamics of the disease. Till date estimation of life span of *W. bancrofti* has been made either from the follow up of microfilaraemia in few individuals, who have moved from endemic to non-endemic area or based on experimental studeis in animals. Here an attempt has been made to estimate the fecundic life span of adult female *W. bancrofti* based on longitudinal follow up of 631 microfilaraemic individuals in Pondicherry for a period of 5 years (1981–1986).

The loss of infection was utilized to estimate the fecundic life span of *W. bancrofti* female by modifying Anderson's model. The rate of change in the infected population (I) with the time (t) is given by

$$dI/dt = -mI$$

Where m is the instantaneous death rate.Then the infected population at time(t) is given by:

$$I(t) = I_{(t=0)} e^{-mt}$$

and the proportion(P) of people infected at time(t) is given by

$$P(t) = I(t)/I_{(t=0)} = e^{-mt}$$

Thus the death rate can be calculated from the proportion still infected

$$m = -LN(p(t))/t$$

The mean expected fecundic life-span of infection is simply the inverse of the death rate (I/m).

The death rate of adult parasite (0.1935) was calculated from the proportion still infected (in this case 38%) after a known time period (in this case 5 years). Hence the mean expected fecundic life span is estimated to be *5.17 years*. It is worth

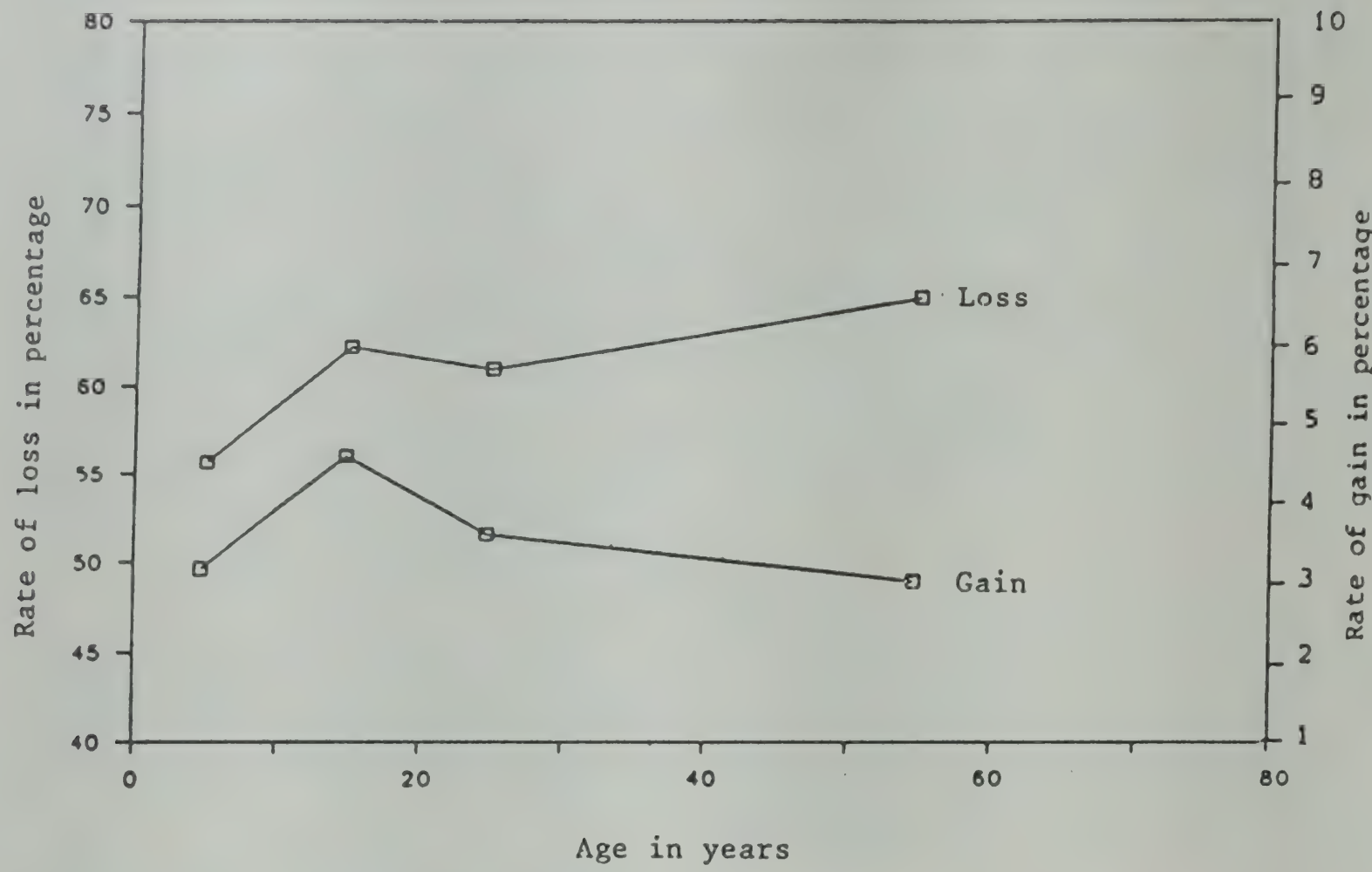


Figure 3.2 Age specific loss and gain in infection over a period of five years.

mentioning that this estimate is based on the data where some of the positives were treated with Diethyl carbamazine.

Therefore the life span was estimated separately for those who received DEC and those who did not receive any chemotherapy. The fecundic life span estimated from the group who did not receive chemotherapy reflects natural loss and was *10.25* years and from those who received treatment was 5.33 years.

To study the change in microfilariae discharged by an adult female worm with age over its life span, the age wise analysis of mF intensity among those positive for mF in both the surveys ($n = 105$) and who have not received any treatment was also done (Table 3.2). The non parametric Mann Whitney U- test showed that the changes were not significant ($P > 0.05$). Since intensive vector control operation was carried out during the study period, fresh transmission, and consequent acquisition of infection is assumed to be negligible. It is worth mentioning that during this period there was more than 80% reduction in both the transmission potential and risk of infection index. This estimation from the longitudinal follow up of a cohort in an endemic area is the first of its kind and obviously more reliable than the earlier reports. Moreover for the first time loss due to chemotherapy and natural loss could be differentiated.

3.1.4. Density dependent factors limiting the intensity of infection in the human host:

The changes in the size of animal population is subjected to (a) births, (b) deaths, (c) immigration and (d) emigration. In the case of helminth parasites the births within a definitive host do not increase the adult parasite numbers. Also no emigration of adult parasites from the host can occur. Hence the adult population of helminth parasites in their final host is being regulated only by immigration and deaths. The life cycle of *W. bancrofti* involves two distinct populations, namely, the adult parasite and microfilaria in human host, and the larval stages in the vector population. The development of microfilaria to L3 (infective for man) takes approximately 10 to 12 days in mosquito. The survival of the parasite therefore depends on the survival of infected mosquito. Hence the acquisition of infection by the human host depends on the survival and the level of infection on the vector.

Prevalence of infection and disease in the population are generally assumed to be a function of intensity of transmission in an area. The most useful measure of intensity of transmission would be the rate of aquisition of adult worms by man, regardless of whether such worms were mated and fecund, or not. However it is not feasible with any available technique to measure adult worm burden. In such a situation if we assume that the rate of microfilaria production is independent of parasite age and host response to parasite infection, then the mean microfilaria count could reflect the number of adult females in the host. The earlier analysis indeed showed that the mF production is stable for atleast a period of five years, therefore the mF production can safely be assumed to be independent of parasite age. Further it was observed that the rate of loss of infection is also independent of host age, which could mean that the loss is purely a function of parasite survival and not influenced by host response to infection. Therefore it is reasonable to assume that mean mF count reflects the adult worm burden in the human host.

Therefore the relationship between worm burden in vector population and human host is studied by correlating infective resting man hour density of vector and mean microfilarial density in human host from different areas. Since earlier analysis showed that the mean intensity of infection and prevalance stabilised at the age classes above 20 years, the mean mF count in adult age classes above 20 years was used.

Assuming that a metazoan infection, like filariasis, is at an equilibrium level in the human population before the onset of effective control measures, the simplest theoretical description of average intensity of infections of humans I(a) at age '*a*' is an Immigration-Death model, described by the differential equation:

$$dI/da = L - MI \qquad \ldots\ldots\ldots (1)$$

Here the net rate of change of intensity (dI/da) with respect to host age is expressed as the difference between the net rate of new infection (L) and the death rate of adult parasites. The net death rate is the product of a per capita death rate 'M' and

TABLE 3.2

Changes in microfilaraemia in 105 persons in Pondicherry over five years

Age group (years)	Persons remained positive	No. with increased mF	No. with decreased mF	No with mf un-changed	Mean Mfc 1981	Mean Mfc 1986	MANN Whitney U test	One tailed P value
1–10	3	3	0	0	1.67	7.00	1.31	0.09
11–20	34	18	14	2	8.18	11.80	0.78	0.22
21–30	24	13	10	1	16.25	16.67	0.24	0.41
31–40	16	8	6	2	8.06	14.81	0.66	0.25
41–50	17	12	4	1	11.24	17.11	0.96	0.17
>=51	11	7	4	0	10.91	15.82	1.31	0.09
Total	105	61	38	6	10.60	14.51	1.95	0.02

"Pollution must be brought under control and mankind's population and consumption of resources must be steered towards a permanent and sustainable equilibrium. 'Unless this is done, sooner or later — and some believe that there is little time left — the downfall of civilisation will not be a matter of science fiction. It will be the experience of our children and grandchildren.'

But how is it to be done? What are the 'moral choices'? Is it just a matter of deciding 'how much we are willing to pay for clean surroundings?'"

— E.F. SCHUMACHER (1973)

in *"Small is Beautiful"*

the adult intensity *I*. This equation describes solely the adult parasite population in a single host and has a standard solution,

$$I(a) = L/M\,(1-e^{-Ma}) \quad \ldots\ldots\ldots (2)$$

which is simply the age intensity curve (intensity $I(a)$ as a function of age, ('*a*') of infection. This relationship shown in fig. 3.3 indicates an initial increase in aquisition in the younger age classes (when the accumulation of new infections predominates over parasite deaths) followed by a plateau in the adult age classes, when aquisition of new infection is balanced by parasite death. The maximum intensity is therefore obtained by equating $dI/da = 0$ and solving for I we get,

$$I_{max} = L/M \quad \ldots\ldots\ldots (3)$$

This would indicate that the plateau in infection intensity with age is not necessarily a consequence of acquired immunity, since the establishment (*L*) and death (*M*) rates of infection are constant, and are not assumed in the model to be affected by the hosts experience of infection. If the equation (3) is used to plot the maximum infection intensity (I*max*) against the infection rate 'L' (fig. 3.4) a straight line would result if I*max* is proportional to the infection rate in the vector. By contrast, if any density dependent factor is operating, it is expected that the I*max* would reach a ceiling at high infection rates in vectors, or even to decline (these cases are illustrated in fig. 3.4).

Only the 1981 data set was used since this data was not affected by the impact of intensive vector control measures in Pondicherry undertaken by VCRC. The principal vector of bancroftian filariasis in Pondicherry is *Cx. quinquefasciatus*. Resting and biting collections were made through out the project period 1981–85. Resting collections were done from 17 fixed stations every fortnight and biting collections from 5 sites for every week. Of these 17 sites 5 are common to both biting and resting collections. In order to have more data points for the comparison of parasitological data with entomological variables it was decided to use the resting data provided there is a linear relationship between biting and resting collections.

An analysis was done to correlate both biting and resting per man hour densities from the common sites of biting and resting and it was found that resting density was linearly related to biting density ($r = 0.94$, $P = 0.00$, $Df = 46$) except site 5 where no corelation was found and hence was omitted. As a result the infective resting density (IRD), *i.e.* proportion of resting mosquitoes with L3 larvae, was adopted as an index of potential infective pressure.

For the application of the model mean microfilaria density in adult human population from 1981 survey data for the corresponding 16 sites were included.

Mean microfilaria density in human host (Fig. 3.5) shows a significant increase and then decline with IRD (overall polynomial fit $P < 0.05$). The fitted curves represent best fit least squares polynomials which, in this analysis, are used to illustrate trends in the data. A comparison with fig. 3.4 shows that this convex pattern of microfilaria density with infection rate in vector population indicates that some density dependent factor or factors limit the acquisition of infection by human host at high transmission intensities. The prevalence of mF in adult with IRD relationship (fig. 3.6) indicates a broadly similar pattern, although the prevalence data are rather more variable. One important point to emerge from fig. 3.6 is that a marginal reduction in infective resting densities may not reduce infection prevalence significantly until a high level of control is reached.

Adult infection intensity in humans at first increase and then decline with infective mosquito resting density. This finding, provides circumstantial evidence for the operation of density dependent regulating factors in lymphatic filarial infections of humans. Identifying and elucidating such factor is central to a quantitative understanding of filariasis and its control. Further analysis is to be carried out to produce an explicit theoretical model of the effects of individual density dependent factors responsible for limiting the parasite establishment at higher infective vector density.

3.1.5. Impact of human population density on filarial transmission:

It was observed that the filarial transmission is also regulated by the density of human popula-

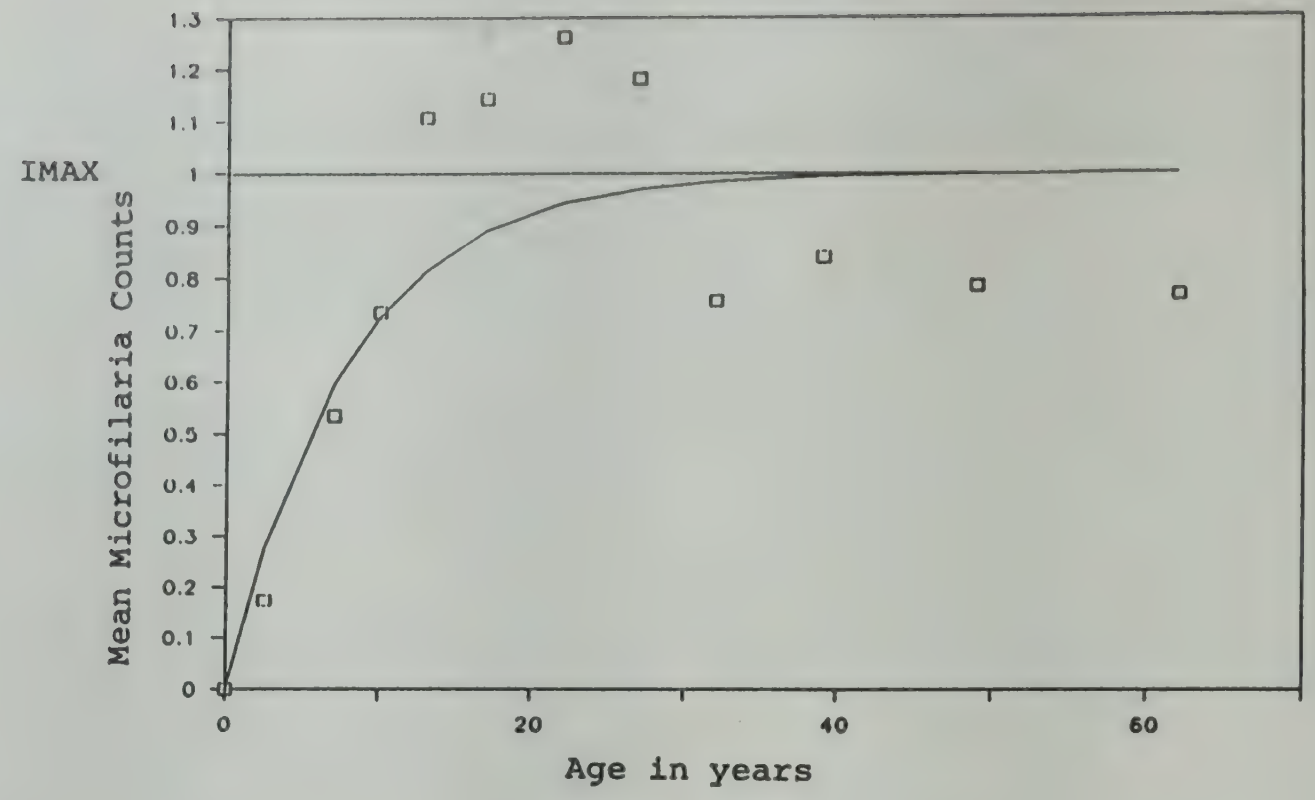


Figure 3.3 The mean microfilaria density as a function of host age. The equation for the curve is, $I(a) = 1.008\ (1-e^{-0.129a})$

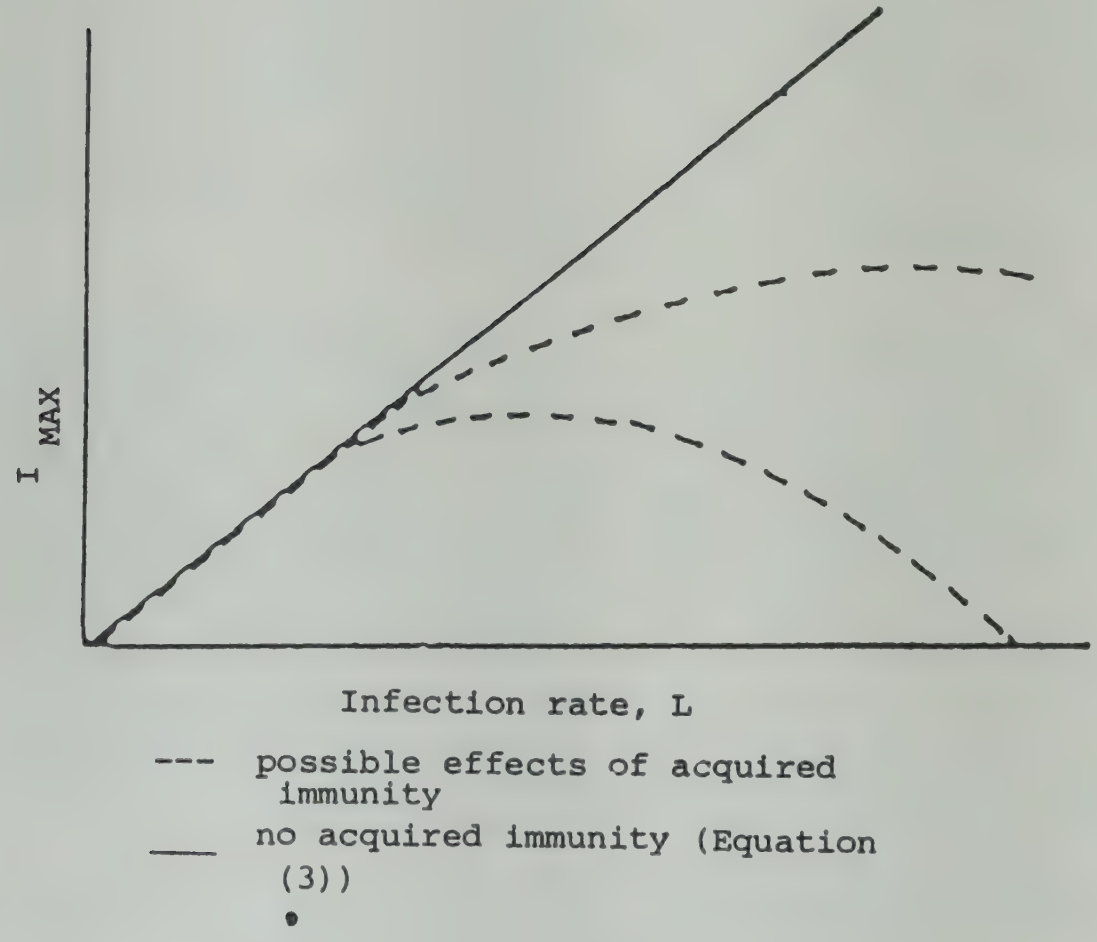


Figure 3.4 A hypothetical graph showing the relationship between maximum microfilaria intensity in adult humans and infection rate in vectors.

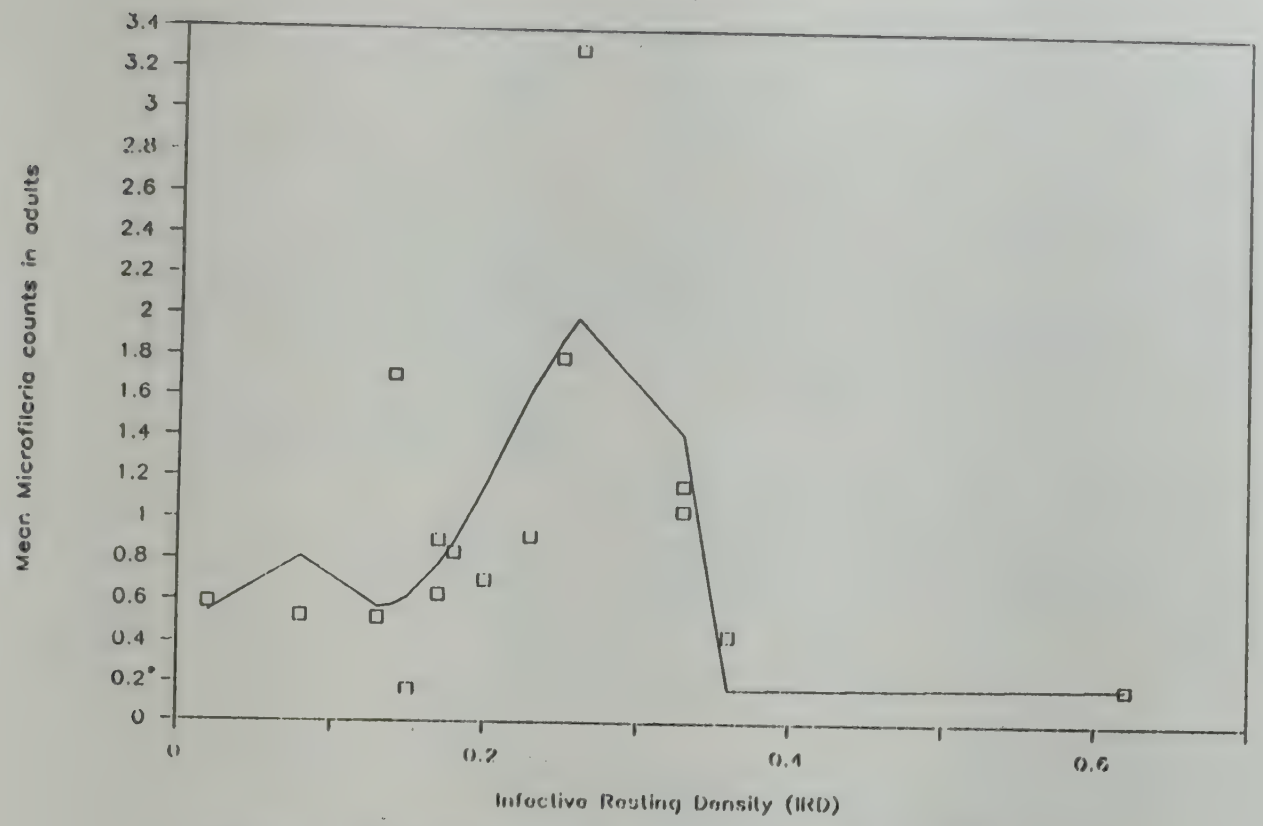


Figure 3.5 Relationship between IRD and mean micro-filaria density.

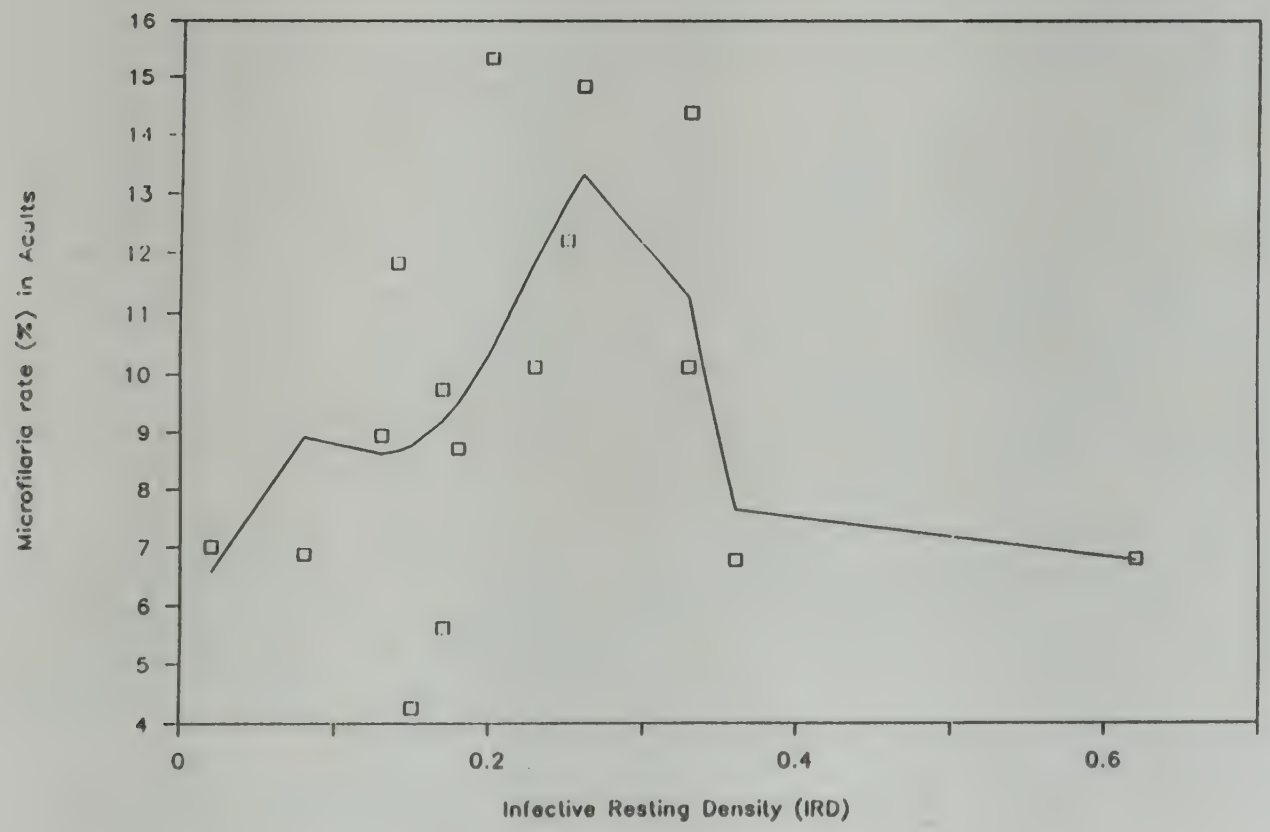


Figure 3.6 Relationship between IRD and microfilaria rate (%) in adult humans.

tion. Therefore the data on the mF prevalance and infective resting vector density from different areas with different human population density (per sq. km) was analysed to depict the relationship if any exists. Apart from the 16 sites of urban Pondicherry. mF prevalences, mosquito densities and human population densities from 4 rural sectors of outside Pondicherry were also utilised for the comparison with the more densely populated areas of Pondicherry.

The relationship between mosquito resting density and human population density for the areas of urban and rural Pondicherry district is illustrated (fig. 3.7) by a saturation curve of the form given by least squares fit ($A = 9.5$; $R^2 = 0.487$).

$$\text{Mosquito density} = A/B[1\text{-}e^{-B(\text{Human density})}]$$

Overall, mosquito density increased rapidly with human density upto around 4000 people per Sq. Km. and then appears to reach a plateau. Human population density is likely to affect mosquito density both directly in terms of feeding and indirectly due to the creation of potential breeding sites by the community. The saturation in mosquito density with human density probably reflects a limitation on the density of breeding sites. At low human population densities (below around 500 per Sq. Km. from fig. 3.7), the vector population disappears, reflecting its status as an essentially urban ectoparasite.

Fig. 3.8 illustrates the relationship between average microfilaria prevalence in adults and human population density. This relationship again shows a rise and then levelling off in average infection prevalence as a function of human population density, with some evidence of a fall-off in prevalence at high densities. The fitted relationship is

$$\text{Prevalence} = A/B\ [1\text{-}e^{-B(\text{human density})}]\ [e^{-C(\text{humandensity})}]$$

$$A = 6.3, B = 0.5, C = 0.025; R^2 = 0.526.$$

There is again (as would be expected) some evidence of a lower threshold of human density for persistence of microfilaria prevalence, but the situation is less clear than that for mosquito density (fig. 3.7). The *upper* limit on microfilaria prevalence is best explored in terms of entomological variables.

The conclusions emerging from the above analysis are that both mosquito resting densities and microfilaria prevalence are related to human population density and that there appears to be a human density threshold around 500 per Sq. Km. in this region. Secondly the relationship between microfilarial prevalence and mosquito density indicates that large reductions in vector density by control measures may be required before the results are apparent in terms of adult prevalence (the effects of vector control on prevalence in younger people are, of course, likely to be much more marked).

3.1.6. Estimation of Biting density from the emergence data:

Biting density is an important entomological parameter used for studying the dynamics of transmission. However, man biting collection from the large number of areas require enormous man power thereby create logistic problem. If the emergence data obtained by the routine larval monitoring team can be used to estimate the biting density, it could solve this problem by providing additional data points. Therefore regression analysis was done to find out the relationship between adult emergence, resting and biting density. Correlation between the total emergence and the resting density was found to be very low for all the zones tested and are not significant ($r = 0.17$, $P = 0.58$, $Df = 10$). However the correlation between total emergence and biting density was highly significant ($r = 0.93$, $P = 0.00$ $Df = 10$). Using this correlation biting rate for the other areas can be calculated. The regression equation is

$$\text{Bitting density} = 1.02 + 0.00001\ (\text{Total Emergence}).$$

Chi square test was applied to test the goodness of fit and the test accepts the hypothesis that the regression line is good fit. Therefore the equation can be used to estimate the biting density from emergence data.

3.1.7. Impact of survival rate of vector on infection rate:

Infection rate i.e. number of mosquitoes picking up infection from the human host is an important factor in predictive models. However,

Figure 3.7 Mosquito Vs. Human Pop. Density

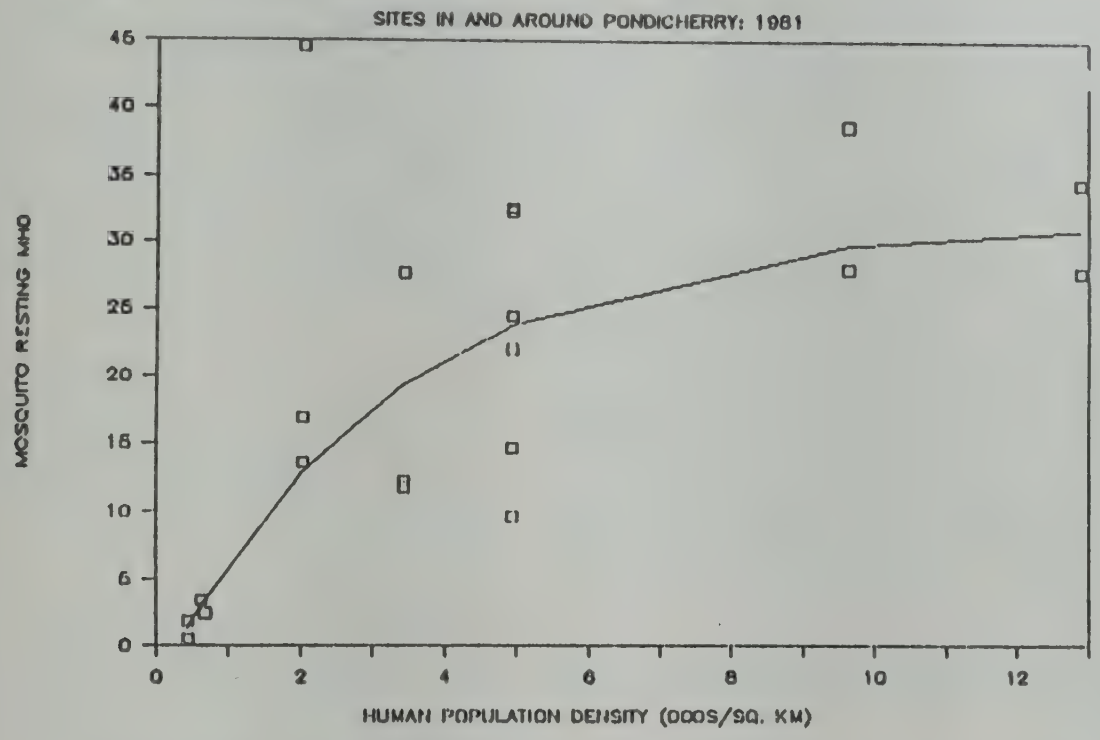
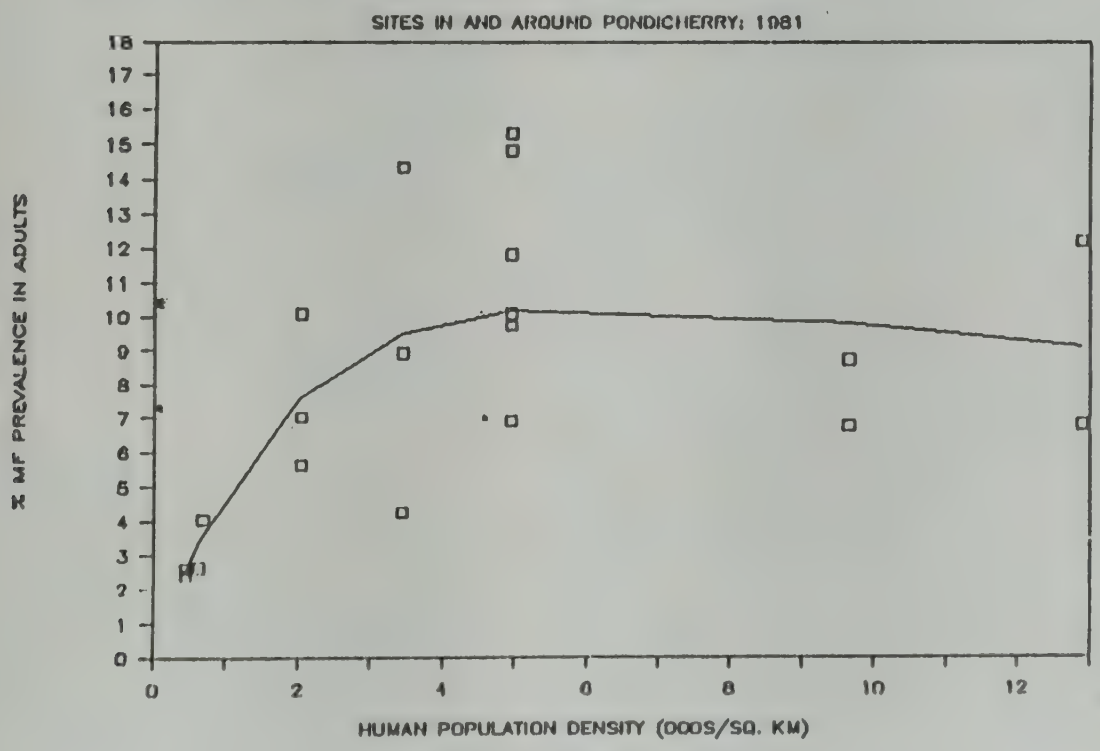


Figure 3.8 Mf. Prev. Vs. Human Pop. Density



in filariasis the parasite undergoes 3 moultings to become infective. Since the infection rate is calculated considering all stages, it is logical to assume that the infection rate will be affected by the survival of mosquito.

When survival rate of mosquito and infection rate in vectors was analysed, there was a significant association between the two. ($r = 0.24$, $P = 0.02$, $Df = 88$). However the statistical significance of low correlation may be due to the large sample size. But there was no significant association between survival rate and infectivity rate of vector mosquitoes ($r = 0.13$, $P = 0.22$, $Df = 88$) indicating that the vector infection is independent of survival. This, however is biologically unacceptable. Hence it is believed that there are other factors which regulate the infection rate in vector population. Otherwise, more sophisticated statistical methods have to be developed to define the exact relationship.

3.1.8. Relationship between Human and vector infection:

It was logical to assume that the infection rate in the vector population reflects the prevalance of microfilaraemia in human population. Therefore the relationship between the infection rate in mosquitoes and mF rate in human population was analysed. It was found that no significant correlation exists between the two parameters. ($r = 0.34$, $P = 0.18$, $Df = 15$ for all 17 LEZs in 1981).

To exclude the survival factor in the usual infection rate in vectors, only mF positive vectors were considered (mF infection rate). Mf infection rate is expected to be linearly related to mF prevalance in human population if the migration is limited and vector mosquitoes were feeding randomly. However the analysis showed no such relationship existed ($r = 0.39$, $P = 0.12$, $Df = 15$, for all LEZs in 1981). This is probably due to clustered distribution of mF carriers in different localities and differential feeding behaviour of the vector mosquitoes.

3.2. Experimental studies on filariasis:

The analysis of FCDP data showed that (i) drastic reduction in vector density alone can eliminate parasite only if such control pressure is maintained for prolonged period, and, (ii) Diethyl Carbamazine (DEC) reduces the fecundic life span of adult worm from 10.25 years to 5.17 year. Therefore it is necessary that the parasite load in the community should be reduced to hasten the rate of decline. However, it is well known that DEC regimen is not readily acceptable to community and in the absence of any other effective drug, an alternative regimen and method of drug administration is called for. Therefore VCRC carried out studies to evaluate the efficacy and acceptibility of selective and mass chemotherapy in two communities and the results are summarised below.

3.2.1. Effect of mass chemotherapy on filariasis

In Kottakuppam a segmemt of isolated population (2232) was selected for mass drug administration. A pre chemotherapy mass blood survey was carried out in this village in which 1,215 persons (55.94%) were examined and 63 were found to harbour microfilariae. Mass administration of DEC was done in the month of January–March 1988 and a total of 1.939 persons (89.2%) were administered with a single dose of DEC at the rate of 6mg per kg body weight. For children below 5 years of age DEC was administered in a syrupy base. The second mass blood survey was done after an interval of 6 months after mass drug administration. 576 blood smears were collected randomly from the study population and 35 of them were found positive for microfilariae. After the second blood survey another round of chemotherapy was done and 1803 persons (82.67%) were treated.

The results indicated that there was a marginal rise in the mF rate from 5.19% to 6.08% (Statistically not significant, $P<0.05$). However, the fall in MFD 50 and mean mF count indicate decreased mF load in the community (Table 3.3). Longitudinal follow up of microfilaraemic individuals showed that of the 45 mF positive persons who were examined in 1st and 2nd survey, 22 (48%) had lost microfilariae. This could indicate that even a single dose of DEC can clear parasitaemia in some individuals. Whether the clearence of microfilariae is temporary phenomenon or permanent is being investigated.

Impact of mass drug administration on the

TABLE 3.3

Summary of Kottakuppam DEC mass Chemotherapy

	1st Survey	2nd Survey
1. Duration of the survey	14-1-1988 31-3-1988	1-7-1988 28-7-1988
2. Population	2172	2181
3. Drug administered	1939	1803
% coverage	89.2	82.67
4. Blood Smears Examined	1215	576
%coverage	55.94	26.41
5. mF Rate	5.19	6.08
6. mFD50	3.59	2.58
7. Mean mF count	0.60	0.52

TABLE 3.4

Pre-control and Post-control microfilaraemia

	Pre treatment	Post treatment 1 month after	Post treatment 1 year after
No. Surveyed	7951	445	1174
No. Positive	930	26	72
mF rate (%)	11.7	5.84	6.13
Annual incidence (per 1000 population)	6.79	3.36	2.83
Mean mF count	2.26	0.67	0.87
MFD–50	7.58	3.48	4.3

mF– microfilaria, MFD-50: Median microfilarial density

8

transmission dynamics was studied by monitoring infection and infectivity rates in vector population. Results showed that immediately after mass drug administration there was a sharp fall in both infection and infectivity rate and after five months infection and infectivity rate started increasing indicating that the mass drug administration reduces microfilaraemia load in the population only temporarily. Further studies are in progress to evaluate the long term effect of mass drug administration on transmission dynamics.

3.2.2. Effect of selective chemotherapy on filariasis:

This study was carried out in a large village (Vettavalam) with an area of 6 sq.km and a population of 10,500. All the positive cases detected in night blood survey were treated with DEC at the rate of 6 mg per kg of body weight. A mass blood survey immediately after selevtive drug therapy was done and the positives detected in this survey were also treated. After one year another survey was carried out and the results are presented in Table. 3.4. Though the fall in mF rate and mF density was significant immediately after treatment subsequent surveys showed again a rising trend. Thus the selective treatment is no way advantageous and full course of treatment for 12 days was impossible to be achieved in a community.

3.2.3. Resilience of Vector population after change over of Strategy from IVM (VCRC) to conventional method of NFCP:

Filariasis Control Demonstration Project was in operation for five years (1981–1985) where vector control was attempted by IVM strategy. Since 1986 the control operations were undertaken by the state NFCP by conventional method, but VCRC continued to monitor the change in vector population from two areas to study the resilience and the results are presented here. Resting density and biting density were monitored and depicted in fig. 3.9. The results indicate that the population had already reached the level of stability and continues to fluctuate depending upon the environmental factors. While in Muthialpet area there was an overall decrease in the population, there was an increasing trend observed in Mudaliarpet. This is mainly due to the fact that in Muthialpet area due to developmental activities many *low lying areas* (supporting vector breeding) have been reduced permanently whereas in Mudaliarpet conversion of agricultural land created additional breeding ground.

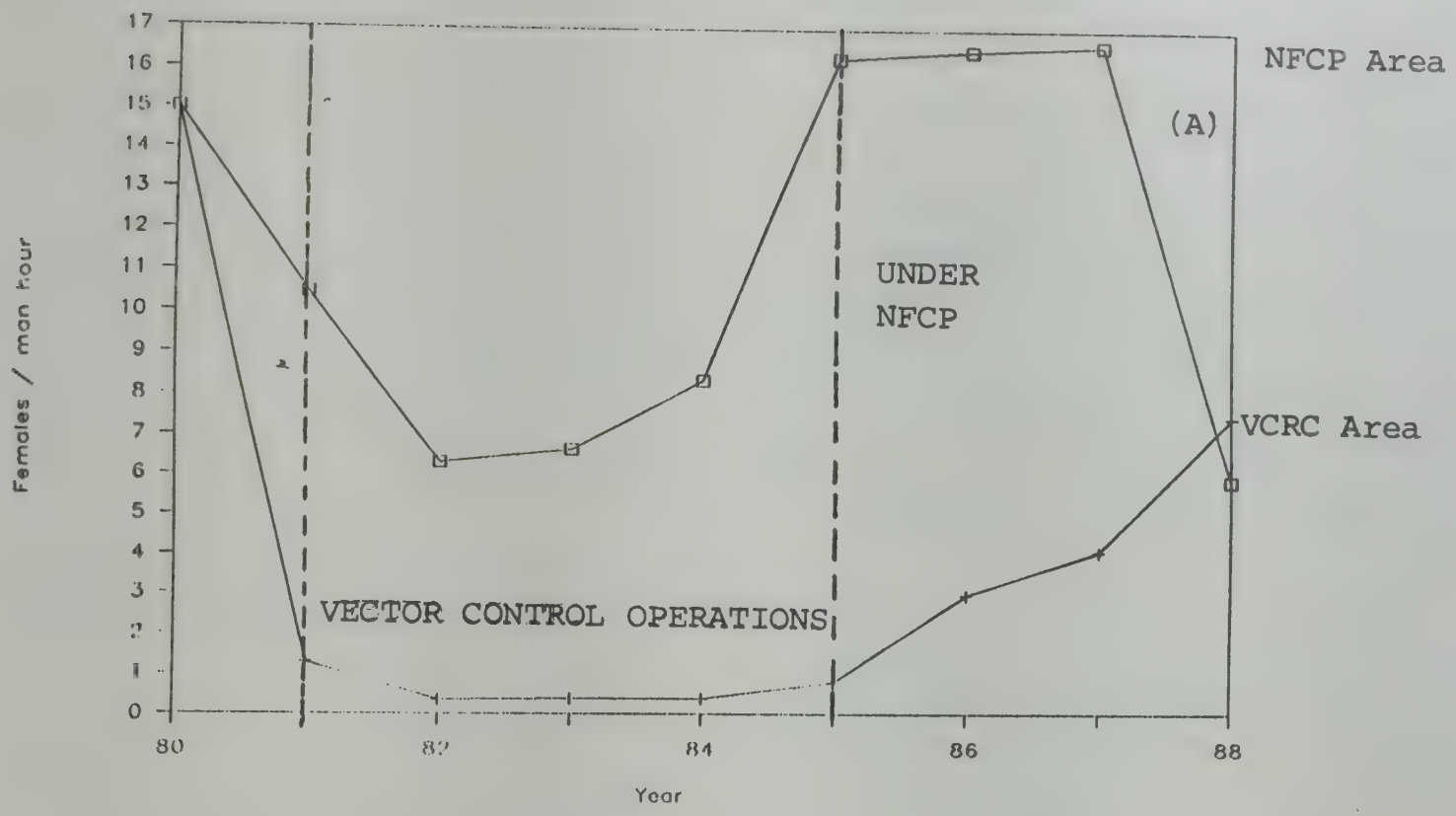


Figure 3.9 Night biting density (A) and indoor resting density (B) of *Culex quinquefasciatus* in the VCRC and NFCP areas during the control period (1981–85) and post FCDP period

4. BIOLOGICAL CONTROL

4.1. Search for Indigenous Insect Pathogens

Twentyseven soil samples were collected from streams, cattle shed, plantations and forest cover in Nilgiris, a hill station in Tamilnadu and were screened for the presence of mosquito pathogens. Totally 17 *B. thuringiensis* and 10 *B. sphaericus* strains were obtained. When tested for larvicidal activity against *C. quinquefasciatus* larvae none of them was promising, i.e., the Lc 50 values varied from 656 ng/ml to 48000 ng/ml which is too much compared to that of the standard strains.

Fortyfour soil samples were collected from ponds, paddy fields and streams in Koraput, the tribal district of Orissa where malaria incidence is high. Sixteen of them have been processed. They have yielded 5 *B. thuringiensis* and 6 *B. sphaericus* isolates which are yet to be tested against mosquitoes. From these samples, 41 fungal isolates were obtained whose spores when tested on mosquito larvae were found ineffective.

One hundred and twenty one soil samples collected from farmyard manure pits, decaying vegetation, decaying hay and compost (from Pondicherry) and the 27 soil samples from Nilgiris were screened for the presence of actinomycetes. Fiftyeight isolates were obtained from them. These were grown for 10 days on agar and their spores were tested against *C. quinquefasciatus* larvae. But none of them proved toxic.

4.2. Mosquitocidal activity of Metabolites from Fungi and Actinomycetes

The culture filtrates of 167 soil fungi and 72 actinomycetes were screened for mosquitocidal activity. Richard's broth was used for growing fungi and YME broth was used for growing actinomycetes. The fungal cultures were incubated at 25 ℃ for 2–3 weeks. Then, the mycelia were removed by filtration, the culture filtrates were sterilised, diluted 10 times and used for testing the larvicidal activity on *C. quinquefasciatus* larvae. The mycelia were extracted with ethyl acetate, evaporated to dryness, the residues were reconsti tuted in sterile distilled water and used for testing larvicidal activity. Observations for larval mortality were made after 24 and 48 h.

The culture filtrates of 36 fungi exhibited varying levels of activity. Sixteen fungi caused mortality within 24 h while 20 fungi induced mortality after 48 h. Interestingly neither the spores nor the mycelial extracts of these isolates killed mosquito larvae indicating that the larvicidal factors produced by these isolates are extracellular. The culture filtrates were also tested for adulticidal activity as per standard procedures, but none of them proved toxic.

The actinomycete isolates were grown for 10 days in liquid medium and their culture filtrates were tested on *C. quinquefasciatus* larvae for entomocidal activity.

The culture filtrates of 17 actinomycete isolates were toxic to *C. quinquefasciatus* larvae. The Lc 50 values of 12 isolates were as follows: 25–100 ul/ml; (5 isolates), 300 ul/ml and 750 ul/ml (5 isolates) and 1000–1250 ul/ml. (2 isolates) (Table: 4.1).

4.3. Mass Production of Lagenidium SP.

This aquatic fungus has shown much promise in killing mosquito larvae in earlier studies. The strain available with the VCRC is the only tropical strain of this fungus. For routine culturing of this fungus PYGSF medium is used. But, for mass production purpose this medium cannot be used as it is costly. Hence, studies were carried out to select a medium for mass production which would be economical to use and the results of the study are presented.

The fungus was grown on the standard PYGSF medium and in media containing corn starch or wheat flour or barley flour or jaggery or cassava starch or rice flour or rice bran, groundnut cake and sunflower oil for seven and eleven days at 30 $\pm$ 2 °C. The zoosporogenesis in such cultures was tested by placing 10 numbers of 8 mm dia mycelial discs in 25 ml of sterile distilled water overnight.

When grown on PYGSF agar medium for seven days the mycelial discs yielded 19.8×10^4 zoospores/ml, while the 11 day old ones produced

TABLE 4.1

Toxicity of Actinomycetes metabolites against *Culex quinquefasciatus*

Isolate No.	LC 50 (in/uls)
01	75
04	50
05	25
08	500
12	750
31	35
43	400
46	85
48	1250
50	85
54	300
66	1000

TABLE 4.2

Zoopore production and larvicidal activity of *Lagenidium* from different batches of mass production

Batch	No. of P. dishes produced	Zoopores $\times 10^4$/ml	Larvicidal activity (% mortality)
1	500	13.066	62
2	500	12.34	52
3	500	14.93	80
4	300	13.77	58
5	600	13.58	57
6	600	15.32	88
7	1100	14.13	86
8	500	14.42	51
9	375	12.76	66
10	400	13.21	70
11	500	12.58	92
12	500	12.69	68

11.2×10^4/ml (Fig. 4.1). Highest number of zoospores were produced by the fungus when grown on medium containing rice bran. The 7days old culture grown on this medium yielded 25×10^4 zoospores/ml and the 11 days old one produced 22.8×10^4/ml. The cultures of the same age from other 6 media produced 20.1×10^4–20.6×10^4 zoospores/ml. The 11 days old cultures from the media containing corn starch or wheat flour or barley flour or tapioca starch or rice flour yielded 11.1×10^4 zoospores, while that from the medium containing jaggery yielded 21.4×10^4 zoospores/ml. The results show that more number of zoospores were produced by the fungus when grown on the medium containing rice bran, while media containing other carbon sources were as good as the PYGSF medium.

Further experiments were carried out to confirm the results obtained that more number of zoospores were produced by the culture grown on medium containing rice flour (RGNCSF). For this purpose the fungus was grown in PYGSF and RGNCSF broths for 72 h on a rotary shaker at 26 ± 2°C and the mycelial yield and zoosporogenesis were determined.

The weight of the biomass when the fungus was grown in PYGSF medium and RGNCSF medium for 72 h, were 38 g/l and 43 g/l, respectively and the zoospore production was at 1.6×10^4/ml and 6.9×10^4/ml when 100 mg of mycelia were placed in 25 ml of water, overnight (Fig. 4.2). When the fungus was allowed to grow beyond 72 h the biomass yield started decreasing. The results indicate that more quantity of biomass and higher number of zoospores can be obtained if the fungus is grown in RGNCSF broth.

The potential of the RGNCSF medium in supporting better growth and zoosporogenesis was verified further by using it in mass production of the fungus. Twelve batches of mycelia totally employing 6300 petri dishes of 10 cm dia were produced. The zoospore yield from different batches ranged from 13×10^4/ml to 15×10^4/ml (Table. 4.2). The results show that the mycelia obtained from different batches had consistently yielded good number of zoospores.

4.4. Developing Storage Methods for Lagenidium

The fungus was grown on RGNCSF agar medium (Rice flour: 3g; Groundnut cake: 2.5 g; Refined sunflower oil: 10 ml; Agar: 12g; Distilled water: 1000 ml; pH: 7.0) for 7 days at 30 ± 2°C. The mycelia were stored in different storage media as detailed below:

1). Fifty numbers of 75 mm dia mycelial discs were placed in 50 ml of sterile distilled water (pH 7.0) in 12 × 10 cm size sterile polypropylene bags (ppb). The ppb were sealed after removing most of the air and stored. 2). Kaolin and celite were prepared as semisolid blocks (10 × 10 cm) and were autoclaved three times on three consecutive days. Fifty mycelial discs were sandwiched in between two blocks. These blocks were placed in enamel trays and were wrapped with brown paper to minimise loss of moisture. Mycelial discs were placed in petri dishes which served as control. All these materials were stocked in a dark chamber and samples of mycelial discs were drawn at 15 days intervals, checked for zoospore production and assayed for larvicidal activity against I instar *C. quinquefasciatus* larvae.

The mycelial discs (10 nos.) induced 75 per cent larval mortality before they were stored in different media. After storage, the distilled water stored mycelial discs induced 35–78 percent larval mortality upto 135 days; the Celite and the petridish stored mycelial discs induced 58–79 per cent larval mortality upto 30 days only; and the discs stored in Kaolin did not induce mortality after 15 days of storage (Fig. 4.3). The results indicate that the fungus when stored in distilled water remained active for as long as 135 days whereas in other storage media like Celite and Kaolin it was active for only short periods.

Another set of mycelial discs were placed in ppbs containing sterile distilled water adjusted to different pH values and stored as described above. Samples were drawn from this stored materials at 30 days interval and tested for zoosporogenesis and larvicidal activity.

The mycelia stored in distilled water at pH 4 killed 30%, 70% and 33% of the exposed larvae respectively, after 30, 80 and 130 days of storage (Fig. 4.4). Those stored at pH 5 caused 34%, 24% and 61% larval mortality when tested after corresponding period of storage. Those stored at pH 6 and 7 induced 26–28%, 60–76% and 20–40% mortality respectively, after storing for 30, 80 and

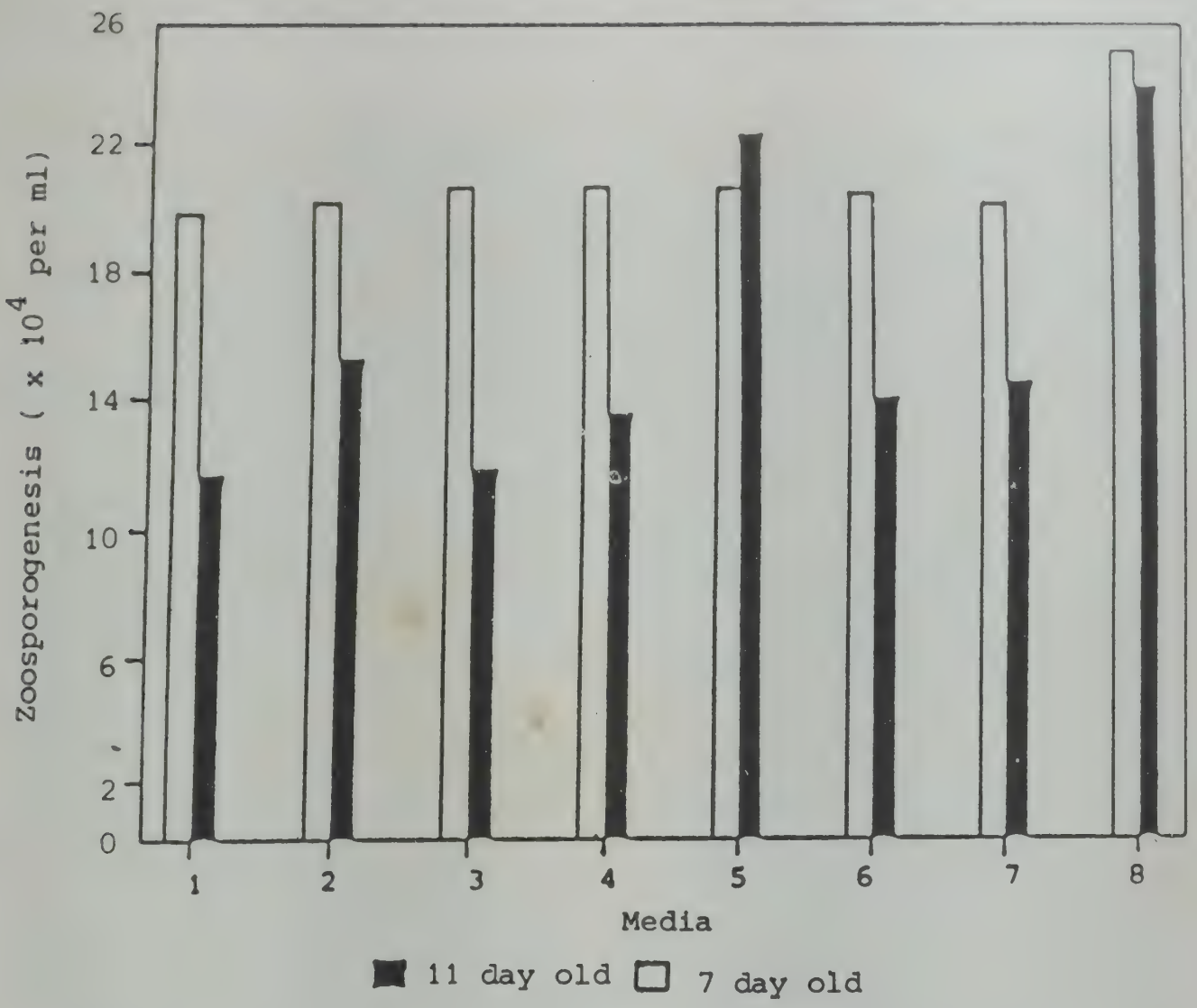


Figure 4.1. Effect of culture medium and age of the culture on the zoosporogenesis of *Lagenidium* (Media: 1. PYGSF, 2. Cornstarch, 3. Wheat flour, 4. Barley flour, 5. Jaggery, 6. Tapiocastarch, 7. Rice flour and 8. Ricebran).

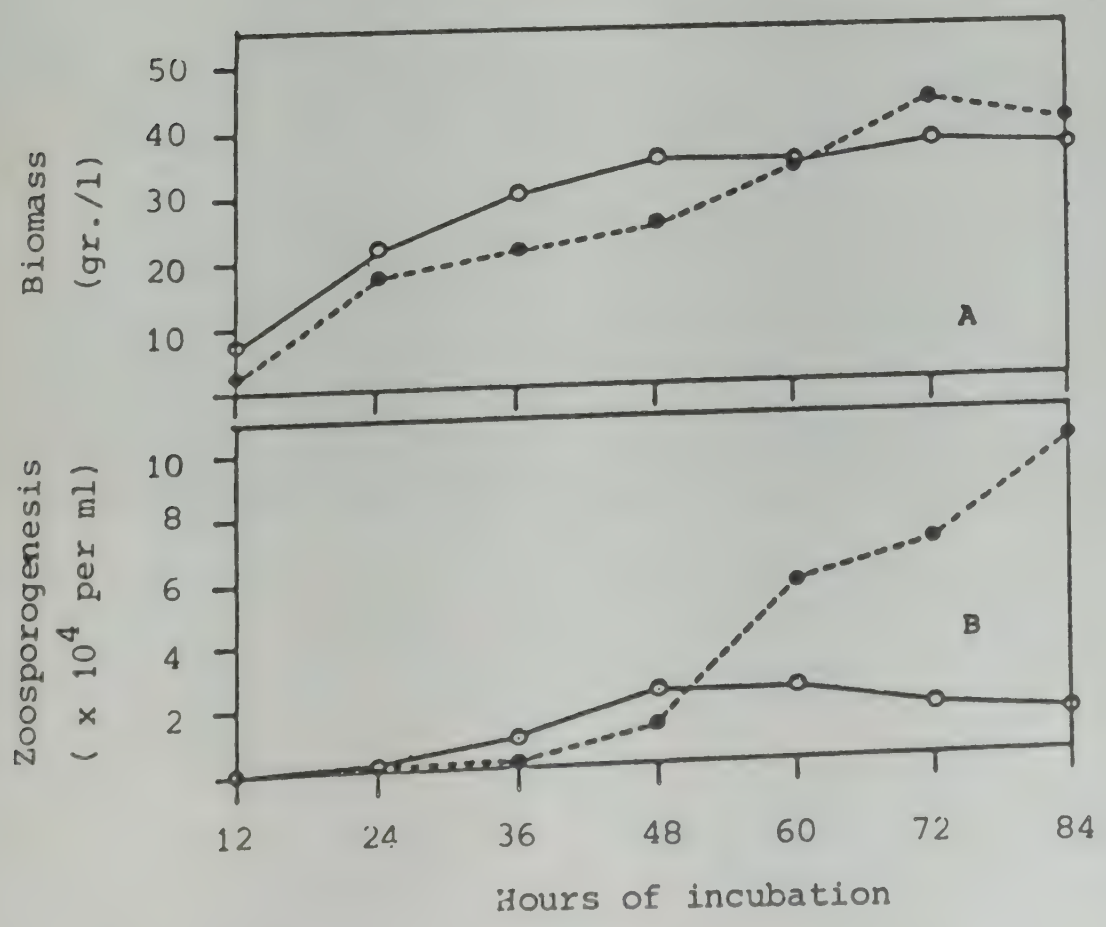


Figure 4.2. Dynamics of growth (A) and zoosporgenesis (B) of *Lagenidium* when grown in PYGSF (○———○) and RGNCSF (●— — —●) broths.

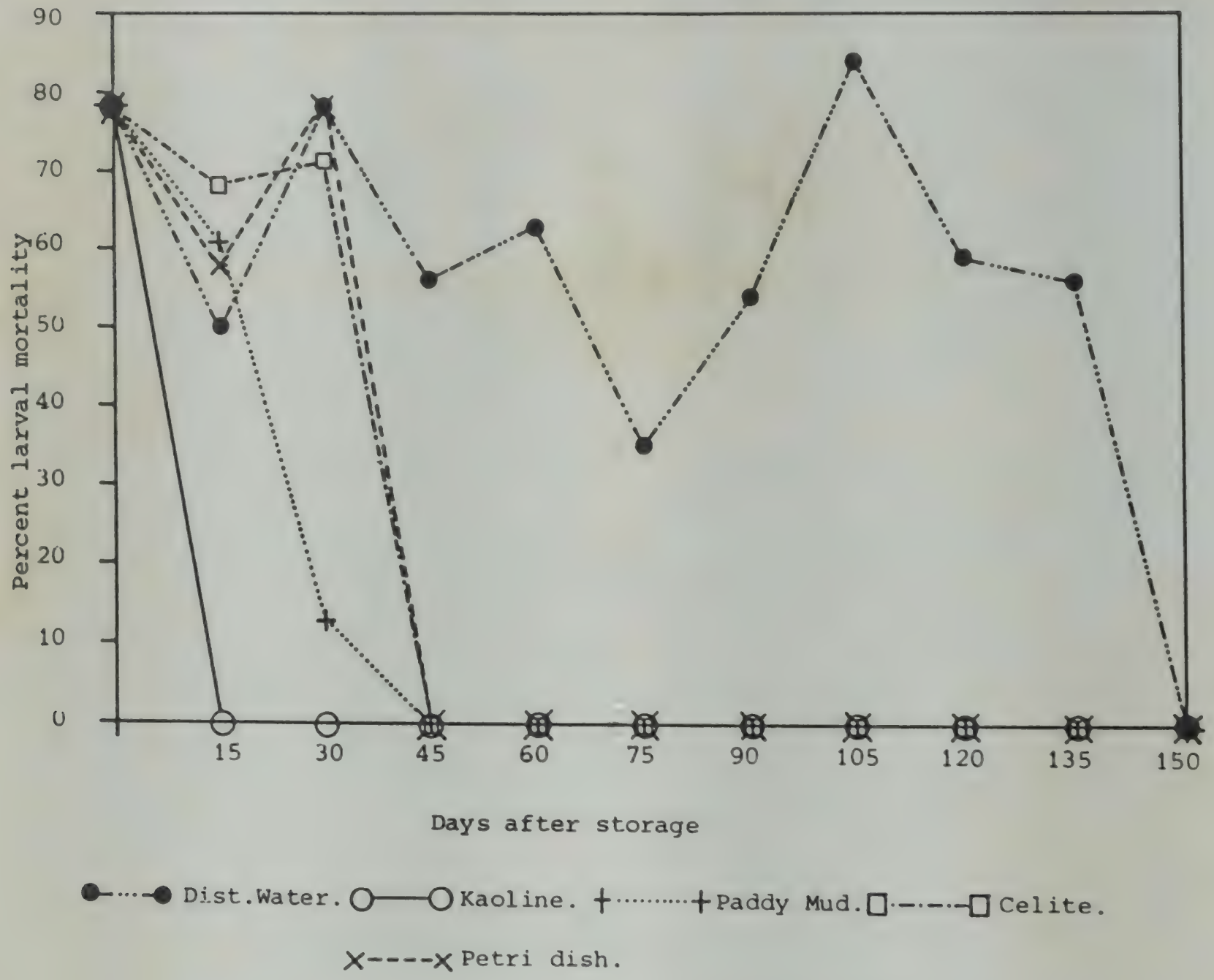
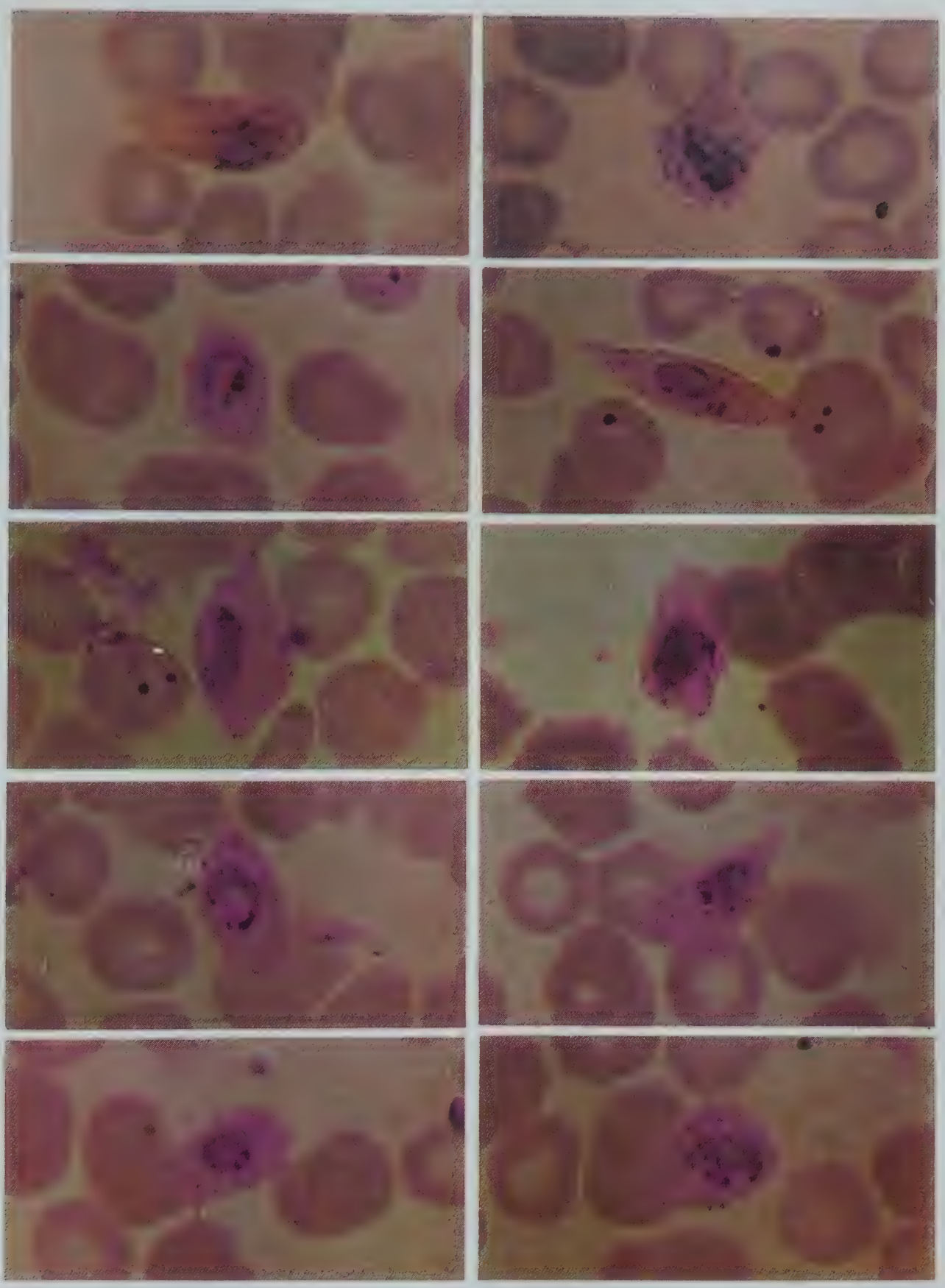


Figure 4.3. Effect of storage media on the larvicidal activity of *Lagenidium* sp.



Plasmodium ovale hitherto recorded from Africa, was found in 7 Patients from two top hill villages, Champapodar and Masipodar in Koraput District, Orissa.

(Please see Section, 2.1, p. 27)

PLATE 2

A

B

Temporary malaria clinic in a remote tribal village, Koraput, showing drug distribution (A) and taking temperature from a fever case (B).

(Please see Section, 2.1.2, p. 32)

A

B

The tribals eagerly await the arrival of the VCRC team for malaria surveillance and treatment—(A) 'Bonda' Tribe (B) 'Kondh' Tribe.

(Please see Section, 2.2, p. 35)

PLATE 4

A

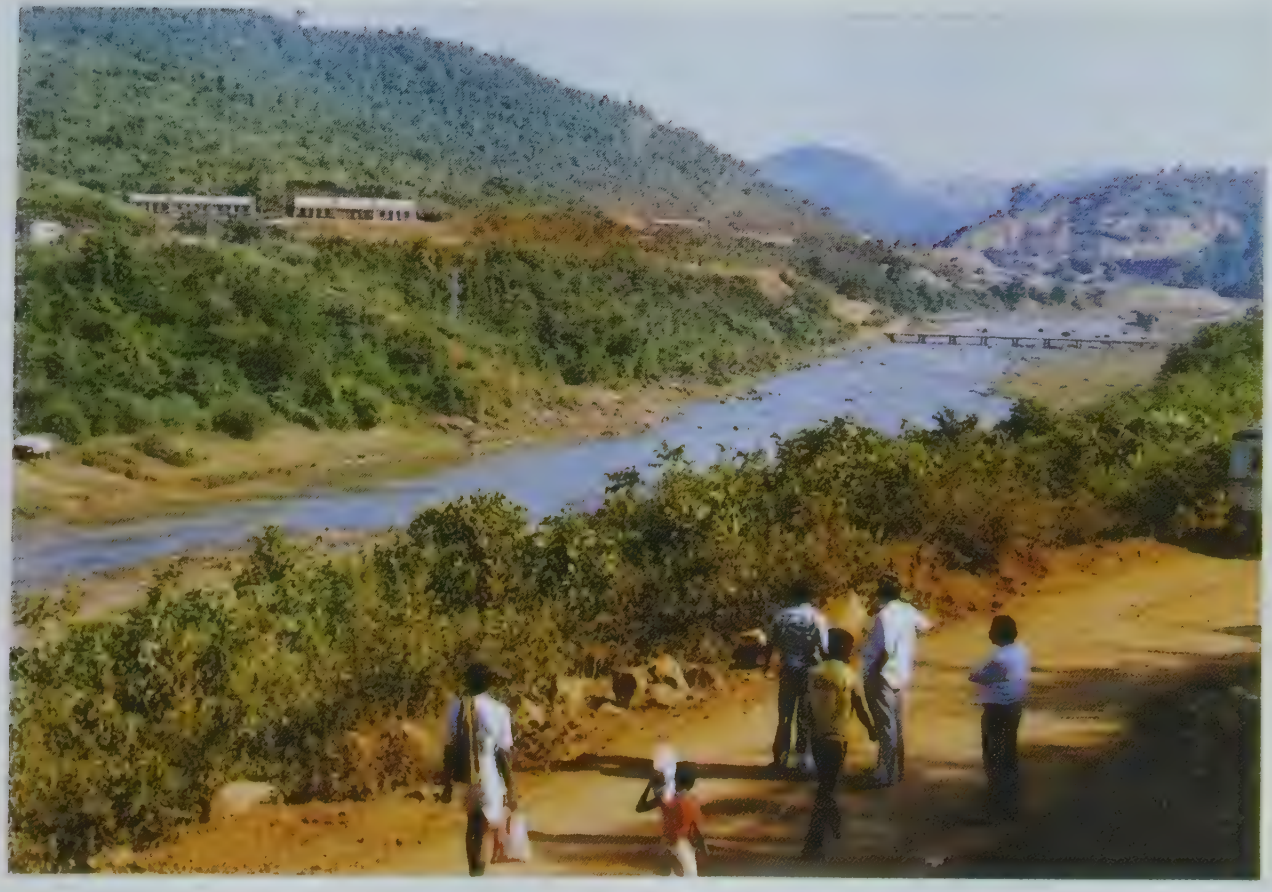
B

A typical KOYA tribal village in Malkangiri, Koraput (A). The tribals depend on forest products for their livelihood and trek many miles a day (B).

(Please see Section, 2.1.2, p. 32)

A

B



Construction of irrigation dam at Muran (A) which attract immigrant labour force. Malaria is the major illness among them. The river Muran (B), the major source of malaria vector breeding in this area.

(Please see Section, 2.3, p. 39)

PLATE 6

A

B

Introduction of salinity in a large tank resulted in drying up of weeds (A) which support Mansonia breeding. Ducks are increasingly being used to control weeds (B).

(Please see Sections, 1.2.2 & 1.5, p. 17 & 18)

A

B

DAINCHA, a leguminous plant (A) is being provided to the people for manuring coconut plants, as a substitute to *Pistia* and *Salvinia*, Root of a Daincha Plant (B) showing nitrogen fixing nodules.

(Please see Section, 1.4, p. 18)

PLATE 8

A

B

The 'FILCO', a voluntary People Organization (B) take active part in clearing weeds from tanks (A) breeding *Mansonia*.

(Please see Section, 1.2, p. 17)

A

PLATE 9

B

Mass drug administration (A) and door to door survey (B) by 'FILCO' Volunteers who actively participate in the *B. malayi* control programme.

(Please see Section, 1.9.2., p. 20)

A

B

Favourite resting place of *Anopheles fluviatilis*—pit shelters (A) and tree holes (B).

(Please see Section, 2.1.3, p. 32)

A

B

Modified "Magoon trap" (A) and Drop nets (B) are used to collect mosquitoes.

(Please see Section, 2.1.4, p. 35)

PLATE 12

A

B

Field evaluation of *Lagenidium* in grassland (A) and in a tree hole (B).

(Please see Section, 4.5, p. 63)

A

B

Field evaluation of *Lagenidium* in seepage pool (A) in rain water collection (B).

(Please see Section, 4.5, p. 61)

PLATE 14

A

B

Evaluation of *Bacillus thuringiensis* H–14 in cess pit (A) and in disused wells (B).

(Please see Section, 4.8, p. 66)

A

B

Evaiuation of *Romanomermis iyengari* in a freshly ploughed paddy field (A) and in a grassland habitat (B) irrigated with sullage water.

(Please see Section, 4.14.5, p. 87)

PLATE 16

A

B

Heavy breeding of *Aedes aegypti* in a cement curing yard of a plant manufacturing Biogas tank (A) and sewage treatment Plant (B) using *Eichornia* as biofilter supporting *Culex quinquefasciatus* breeding. Juvenile Hormone compounds have been successfully used in these habitats.

(Please see Section, 5.2.1., p. 97)

130 days. While mycelia stored at pH 8 and 9 induced low levels of mortality for the corresponding periods of storage, those stored at pH 10 were not at all active against larvae. After 160 days of storage except the mycelial discs stored at pH 7, those stored at other pH levels did not kill the exposed larvae. The data indicate that the optimal level for storage of *Lagenidium* are the pH 6 and 7. While the mortality induced by the mycelial discs stored at pH 4 and 5 were erratic, those stored at pH 8 and 9 have induced low levels of mortality. The result of this experiment indicate that storing the fungus at extreme acidic and alkaline pH is detrimental.

A third set of mycelial discs were placed in ppbs containing sterile distilled water supplemented with traces of glucose, stored and evaluated for zoosporogenesis and larvicidal activity periodically.

The mycelial discs which were washed before storage induced higher level of larval mortality than those which were not washed. The addition of glucose to the mycelial discs which were stored after washing has enhanced the larvicidal activity significantly over those which were stored without glucose. Those stored with glucose caused 54%, 100% and 100% larval mortality after 130, 160 and 190 days of storage, respectively, while those stored without glucose caused 44%, 92% and 6% mortality (Fig. 4.5). The enhanced level of larval mortality induced by the mycelial discs stored in sterile distilled water with traces of glucose may be due to (i) the influence of the glucose on the fungal metabolism, i.e., maintenance of metabolic processess at a lower level in a micro-aerophilic situation, and (ii) the inhibitory effect of glucose on zoosporogenesis. Hence, in this experiment the addition of glucose to the storage medium might have helped to prevent the formation of zoospores during storage.

4.5. Evaluation of lagenidium for larval control in different mosquito breeding habitats

Lagenidium was produced on agar plates for field application purpose using RGNCSF medium. The mycelial discs so produced were tested for zoospore production as well as for larvicidal activity in the laboratory before applying to field. At the time of application the mycelial discs were placed in sterile tap water for 18 h for zoospore production. The water suspension containing the zoospores was sprayed to mosquito breeding habitats with the help of Knapsack sprayers. Paddy fields, and treeholes in Bangalore were chosen for the trial. The paddy field plots measured 400–500 M^2, and the treeholes measured 0.15–0.5 M^2. The paddy fields harboured anopheline and culicine larvae while the treeholes had *Aedes albopictus* larvae. The zoospores were applied at the rate of $9 \times 10^5/M^2$.

Water samples from these habitats were analysed for temperature, pH, alkalinity, conductivity, total solids, total hardness, chlorides and sulphates as per standard procedures. Monitoring of larval populations was carried out prior to the application of zoospores and after the application viz., on 1st, 2nd and 3rd days and then twice a week. Apart from counting, the larvae were randomly examined microscopically for infection. Also, the larval samples collected from these habitats were examined for *Lagenidium* infection by culturing.

The paddy field taken up for field trial was irrigated with water from a tank which gets sewage water from the Bangalore urban area. Chemical analysis of this water showed that it had no dissolved oxygen. The total hardness was 8.6 mg/l, with 78 mg/l chloride and 19.2 mg/l of sulphate. The suspended solid level was 600 mg/l. The data on the larval population in the control and the zoospore treated plots are given in the fig. 4.6.

In the control plot there were 21 early instars and 3 pupae prior to the treatment. Thereafter, upto 34th day the early and late instar populations were encountered on all the sampling days and their numbers were in the range of 8–44 and 6–66, respectively. On all these days, only 6 pupae were found on 4 occassions. In the treated plot, 15 and 7 early and late instar larvae, respectively and no pupae were found prior to the treatment. And on the first, second and third day after application, there were 4 and 12 and 16 and 0 early and late instars, respectively. On 3rd, 5th and 9th days the total larval number was more than what it was prior to the treatment. But on 12th day there were only 5 and 30 early and late instars, respectively and on 27th day there were 10 and 5 early and late instars. No larvae were encountered in the samples collected on 16th, 27th, 30th and 34th days. After

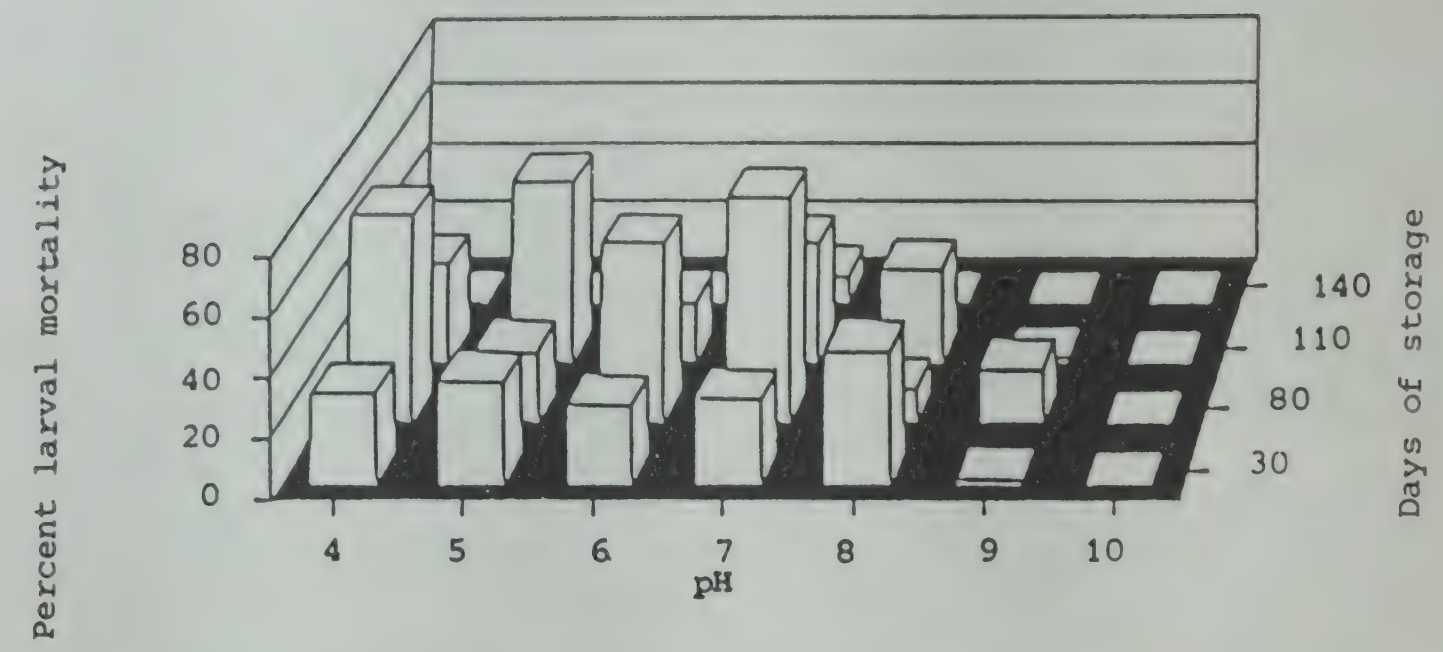


Figure 4.4. Effect of storage of *Lagenidium* in distilled water with different pH levels on larvicidal activity.

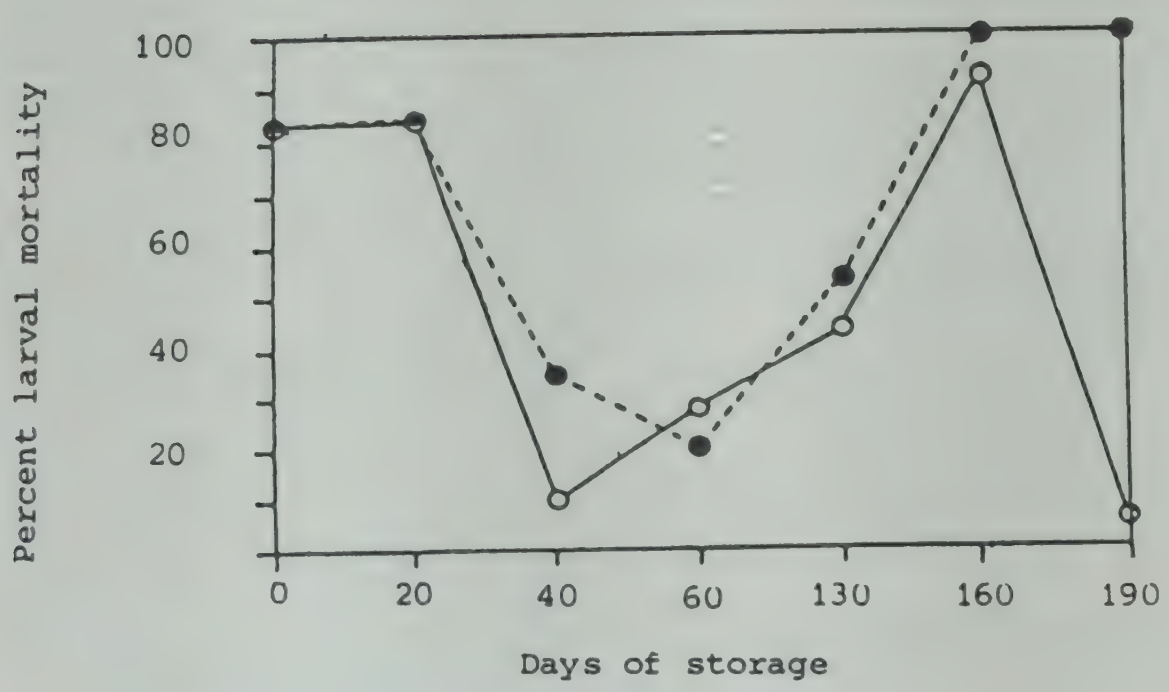


Figure 4.5. Effect of glucose on the larvicidal activity of *Lagenidium* stored in distilled water. (○———○ without glucose, ●— — —● with glucose)

34th day of appliction untill 85th day, larval breeding was observed only on five occasions in the control plots and on 3 occasions in the treated piots. The absence of larvae in the treated plots or presence of larvae in low numbers on many occasions of sampling, when compared with what was observed in the control plots (check area) indicates that the fungus has suppressed the mosquito breeding in the treated plot to some extent. More field tests will have to be conducted in paddy fields to confirm these observations.

Field testing was carried out also in treeholes where the water had 2.8 mg/l of dissolved oxygen. The total hardness was 4.0 mg/l with 15.36 mg/l of sulphates. There were neither chlorides nor suspended solids. In the tree holes which were not treated with *Lagenidium*, there were 21 early instars, 27 late instars and 7 pupae/250 ml of water (Fig. 4.7). From 2nd day to 39th day, 13 samples were taken and during these occasions, these numbers were in the range of 11–76, 20–116 and 5–20 respectively. On 47th, 49th and 50th days, the early instar larval populations increased enormously to 78–355 whereas the counts of late instars and pupae were in the range of 37–105 and 2–11. From 57th to 71st day the larval and pupal counts were comparable with that obtained from 0–39th day.

In tree holes treated with *Lagenidium*, there were 28 early instars, 148 late instars and 69 pupae before application. The early instar population dropped to 5 by 3rd day, increased to 155 by 16th day and from 20th day it declined to 18–83. But the number increased to 144 by 48th day. From 49th to 57th day this number fluctuated between 59–91 and increased to 114 on 61st day. Then there was a sudden drop in the early instar count, with 35,0 and 25 numbers on 64th, 67th and 71st days. The number of late instars and pupae in treated treeholes showed similar ups and downs through out the study period. The results indicate that while in the untreated tree-holes the larval populations fluctuated within normal limits of ups and downs, in the treated treeholes it was not so. In this case, the larval numbers showed steep declines on 4 occasions and this was reflected in the pupal count also. Hence, it is inferred that the application of *Lagenidium* has definitely resulted in significant reduction of the larval density in the treeholes. The observations are being continued.

4.6. Larvicidal factors of Bacillus thuringiensis serotype H14 and H12 isolates

One of the indigenous isolates of *B. thuringiensis* (VCRC B175) was found highly toxic to mosquito larvae. This was serologically identified as H12 serotype. Its toxicity was as good as that of standard serotype H14 strains. Therefore, the present study was taken up to understand the reasons for its high toxicity to mosquito larvae. The BtH14 culture reisolated from the IPS80 standard powder was used for comparison with this H12 serotype. These strains were grown in NYSM broth at 30℃ on a rotary shaker until they lysed completely. The spore-crystal complex was removed and the crystals were separated and purified as per standard procedures. The protein concentration of the crystals was estimated and the toxicity was determined against *C. quinquefasciatus* larvae.

The crystals were solubilised and fractionated by column chromatography. In the case of both the serotypes the toxin fractions were found to elute in 3 peaks. The samples under each peak were pooled and their protein concentration was estimated. The lethality of each fraction, if any, and their Lc50 doses to *C. quinquefasciatus* larvae were determined.

Further analyses have shown that the Peak I mainly contained a doublet with a molecular weight of 130 kDa; peak II contained the 68 kDa protein and peak III had 28 kDa protein. The larval bioassay showed that the peaks II and III were mosquitocidal, the peak II being less toxic than the other. The intact crystals, solubilized crystals, 68 kDa protein and 25 kDa protein had the following Lc 50 values.

Crystal/crystal derivative	H14 serotype ng/ml	H12 serotype ng/ml
Intact crystals	15	11
Solubilized crystals	900	720
68 kDa protein	70	10
28 kDa protein	320	210

Though differences were seen among the two serotypes in the Lc 50 values of the crystal proteins

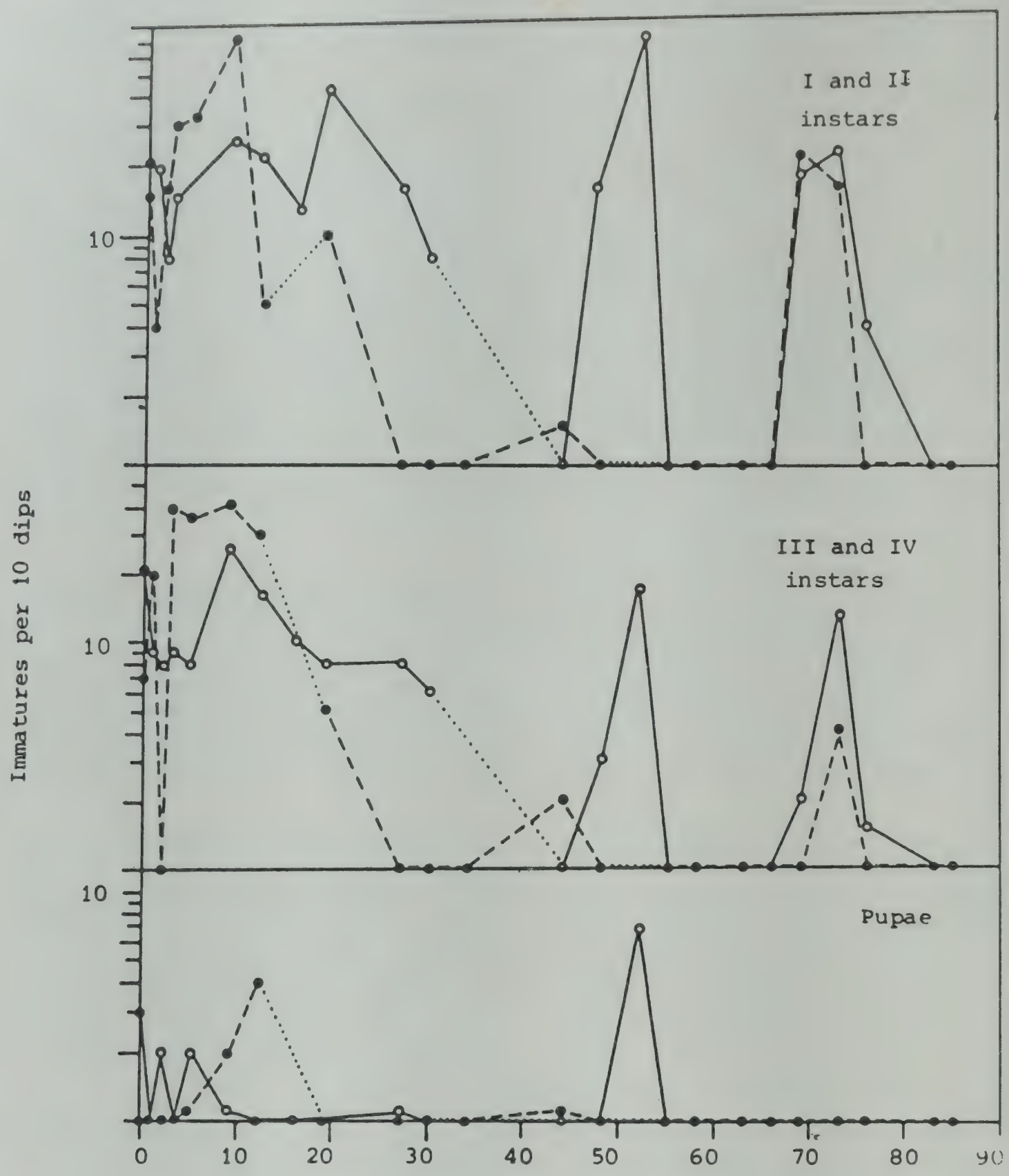


Figure 4.6. Effect of *Lagenidium* sp. on *Culex tritaeniorhynchus* breeding in paddy fields. (○——○ Control and ●— — —● Treated).

●-----● Treated)

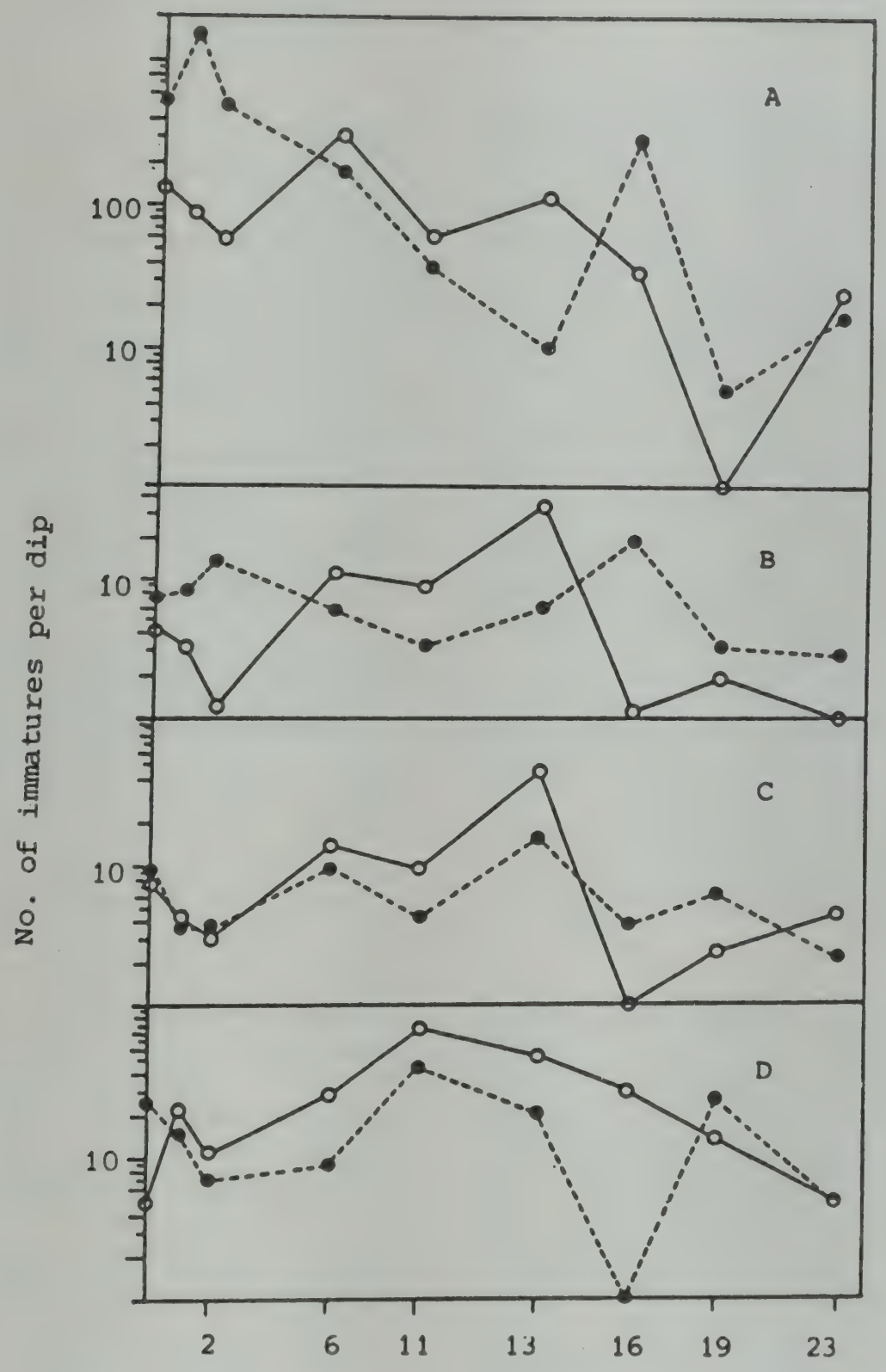


Figure 4.7. Effect of *Lagenidium* treatment on Immature populations (I + II instars-A, III instars-B, IV instars-C and Pupae-D) of *Aedes* sp. breeding in tree holes (○——○ control, ●– — –● treated).

at different steps of purification, practically no difference could be seen in the elution pattern and SDS electrophoretic pattern. Further investigations on the nature of the toxic proteins are in progress.

4.7. Developing cost-effective media for the production of Bacillus thuringiensis H14 and Bacillus sphaericus

Attempts were made to develop cheaper media consituents for the mass production of *B. thuringiensis* H14 and *B. sphaericus*. Soybean seeds were tried as the sole nitrogen source. The seeds were powdered, digested with papain and the liberated amino acids were quantified. The soya digest was incorporated in the production medium so as to provide a final amino nitrogen level of 200 ug/ml. The other constituents of the production medium are D-glucose, 5 g, yeast extract, 5 g, magnesium chloride, 202 mg, calcium chloride, 102 mg, manganous chloride, 10 mg and distilled water, 1 l.

The above medium was dispensed in 200 ml quantities in 1 l conical flasks, sterilized and inoculated with overnight grown cultures at 5%. These flasks were incubated on rotary shaker at 30°C. Samples were drawn from 48th h onwards till 120 h and checked for biomass yield and percentage sporulation.

In the case of *B. thuringiensis* H14 45 g of biomass per l was observed 24 h after inoculation which increased to 50 g/l by 48th h. Thereafer, the biomass level declined and reached 40 g/l by 120th h. Sporulation was observed only after 48 h and reached 90% by 120 h. In the case of *B. sphaericus*, 35 g/l of biomass level was noticed 24 h after inoculation and thereafter the level declined and by 120 h it was at 31 g/l. As in BtH 14, the sporulation began after 48 h and reached 60% by 120 h. When the biomass (120 h) was tested for larvicidal activity on *C. quinquefasciatus* larvae, 100% mortality was observed at 10^{-2} dilution. The data indicate that the medium incorporated with soya digest to serve as the only nitrogen source has supported better biomass production. However, the sporulation of the two bacilli was poor and/or delayed which in turn will badly affect the larvicidal activity of the product. Further studies are in progress to eliminate the problem of poor/delayed sporulation by making suitable adjustments in the carbon:nitrogen ratio of the above medium.

4.8. Evaluation of Bacilus thuringiensis H14 and B. Sphaericus slow release formulations:

The development of a slow release formulation of the larvicidal bacilli using Sodium alginate was reported earlier. This formulation being granular and heavy did not stay floating in the larval feeding zone though exhibited prolonged activity in the laboratory studies. As such this formulation could not be used in deep and larger water bodies. Therefore attempts were made to make this formulation float by placing them in floating containers. This type of *B. thuringiensis* H14 formulation equivalent to 5 Kg a.i. was sent to various scientists in different countries for field evaluation, through the World Health Organization. Also these formulations were evaluated by VCRC in different field stations.

4.8.1. Bacillus thuringiensis H.14

The slow release formulation named as Deltafix was first evaluated in the laboratory for residual activity against *Culex quinquefasciatus* larvae by transferring them every day into fresh set of tubs containing 8 litres of water and 500 II instar larvae along with appropriate controls. Two doses, 500 mg and 1000 mg a.i, were tried. The observations showed that till 24 h there was no significant larval mortality. On 2nd day 71–81% mortality occurred which reached 100% gradually by 28th day (Fig. 4.8). Thereafter till 182nd day the larval mortality fluctuated in the range of 81–100%. From 196th day there was a steep decline in the percentage mortality which reached 3–6% by 213th day. No larval mortality occurred beyond this day. No variations in the % mortality was observed due to different doses and it was assumed that the dose 500 mg a.i. itself was sufficient to cause the expected mortality.

The *B. thuringiensis* H14 formulation was treated to disused and polluted wells in Bangalore, harbouring *C. quinquefasciatus* or anopheline larvae. Totally 36 wells were treated at the dose of 10 Kg/ha. Appropriate controls were maintained. Larval density was monitored for 30 days prior to the treatment and for 155 days after treatment. The samples were taken with buckets. The counts of

III and IV instar larvae and pupae were taken, the means were calculated and expressed as number of larvae per 2 dips. There were heavy rains after 95th day. The data showing the population fluctuation of III and IV instar larvae and pupae are illustrated in figures 4.9 and 4.10.

Anophelines: In the control wells the III and IV instar populations and pupae fluctuated from 1–26, 4–51 and 1–6, respectively during the pre-treatment period with no counts on 2 occasions of sampling (Fig. 4.9.). During the post-treatment period these numbers fluctuated between 1–21, 1–72 and 1–10, with no pupae on six occasions. In the treated wells the III and IV instar population was in the range of 10–39 and 6–42. respectively, during the pre-treatment period. Significant reduction in the population was noticed by 3rd day after application when 6 larvae of each instar were encountered. Between 3rd and 15th day after application these numbers fluctuated between 1–9 and 1–14 with no IV instar larvae on four occasions. There were 2–7 pupae during the pre-treatment period. During the post-treatment period upto 155th day<1 pupa was found in the treated wells.

Culex quinquefasciatus: In the control wells, during the pre-treatment period, the III and IV instar and pupal numbers were in the range of 16–128, 17–114 and 3–23 respectively (Fig. 4.10). And during the post-treatment period these numbers were in the range of 4–552, 3–1185 and 1–177. Pupae were encountered on all the occasions of sampling. In the treated wells significant reductions were observed in the III and IV instar larval population, 4 days after applications. From 4th to 155th day after application the III and IV instar larval numbers were in the range of 13–184 and 17–110 respectively. There were 10–39 pupae during the pre-treatment period and substantial reduction in the pupal number was noticed 8 days after application of *B. thuringiensis* H14. From 8th to 155th day after application the number fluctuated between 2–16 except on 18th day (40).

4.8.2. Bacillus sphaericus:

The slow release formulation of *B. sphaericus* was evaluated against *Mansonia* sp. breeding in ponds heavily infested with aquatic weeds like *Pistia, Salvinia* and *Eichornia.* Surface area of water in the ponds varied from 32–75M² with a mean of 56M². The quality of the water present in these ponds is as follows: Suspended solids: 90 mg/l, Dissolved solids:107 mg/l, pH: 6–7, Salinity: 108 ppm, Dissolved oxygen: 0.64 mg/l. As the data indicate, the ponds were highly polluted due to the retting of coconut husks for making coir. Three Ponds were kept as control and three ponds each were treated with the dosages of 5,10 and 15 kg a.i. per ha. Monitoring for larval number was done at regular intervals, different stages were counted and released back into the ponds. The mean number of larvae present at a given time are expressed as number per 0.3 M². For convenience the data concerned with only III and IV instar larvae are presented.

In the untreated sites, there were 55 to 148 larvae during the pre-treatment period and 39 to 122 larvae during the post-treatment period except on the 33rd day, when there were only 9 (Fig. 4.11). In the sites treated with 0.5 kg/ha, there were 12–90 early instar and 62–186 larvae during the pre-treatment period. Within 24 h after application, there was significant reduction in the larval population wherein 10 early instars and 29 late instars were encountered. From 1st–33rd day after application, the early instar number varied from <1–16, and the late instar number varied from 4–71. The experimental sites being used for retting coconut husk, were disturbed on 34th day after application for the removal of the coconut husk, and therefore further observations were discontinued. The data obtained after *B. spaericus* treatment (from 1st–33rd day indicate that the slow release formulation has released effective dose of the active ingredient to cause significant reduction in the *Mansonia* larval population. Similar results were observed at the higher doses tested indicating that the 5.0 kg/ha dose itself is more than sufficient to exert effective larval control. The field trial is being repeated.

4.9. Antibiotic sensitivity and larvicidal activity of Bacillus sphaericus mutants

Attempts were made to improve the larvicidal efficacy of *B. sphaericus* through classical mutagenesis. This has yielded a few stable mutants which were reported earlier. These mutants were tested for antibiotic sensitivity in comparison with the wild type strain, VCRC B42. The mutants exhibited wide variations in their sensitivity to different antibiotics as shown in the Fig. 4.12.

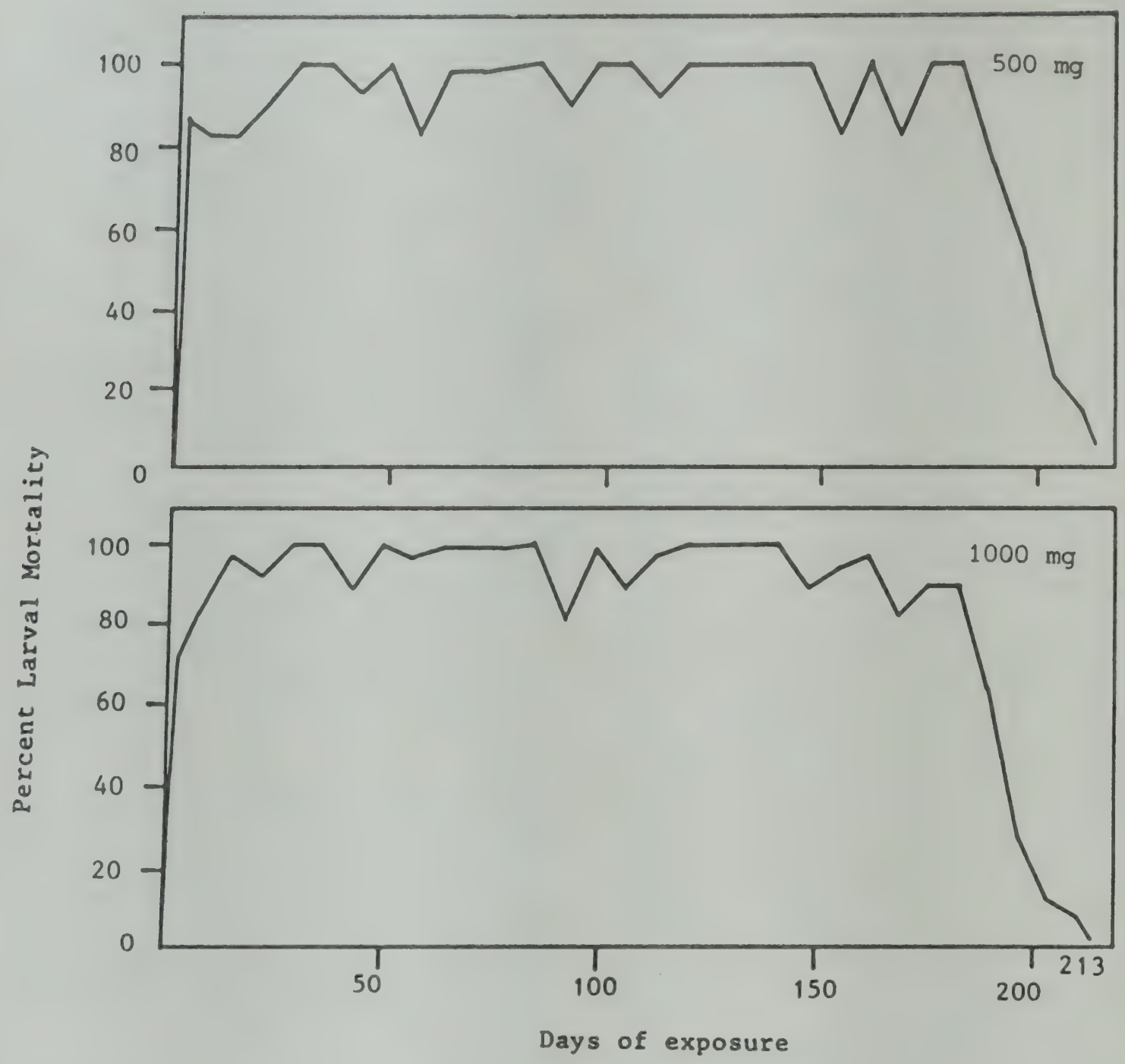


Figure 4.8. Larvicidal activity of Deltafix, a slow release formulation of B.t H14 on *Culex quinquefasciatus* Larvae in the Laboratory.

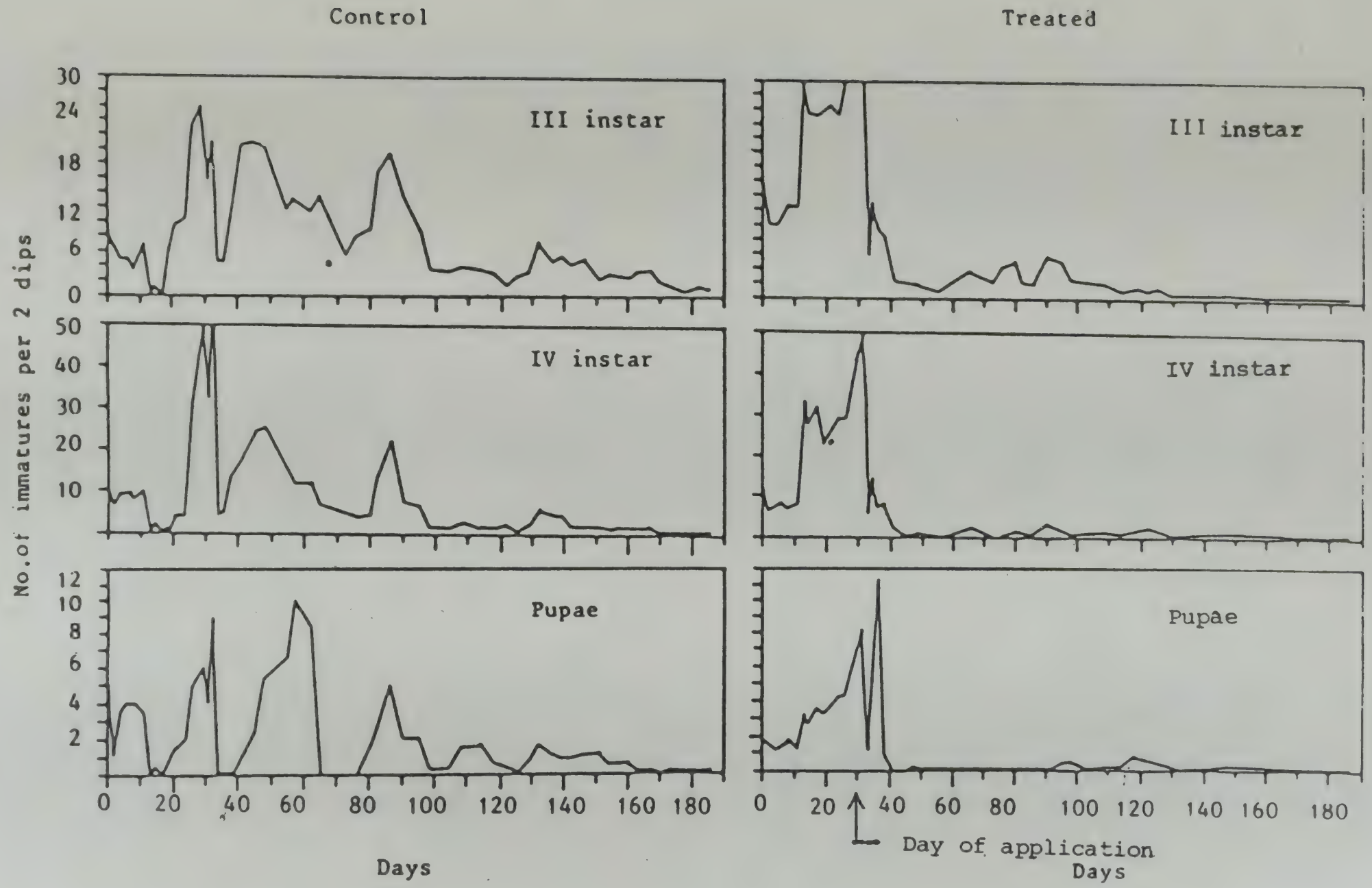


Figure 4.9. Larvicidal activity of Deltafix, a slow release formulation of B.t H14 on immatures of anophelines breeding in wells.

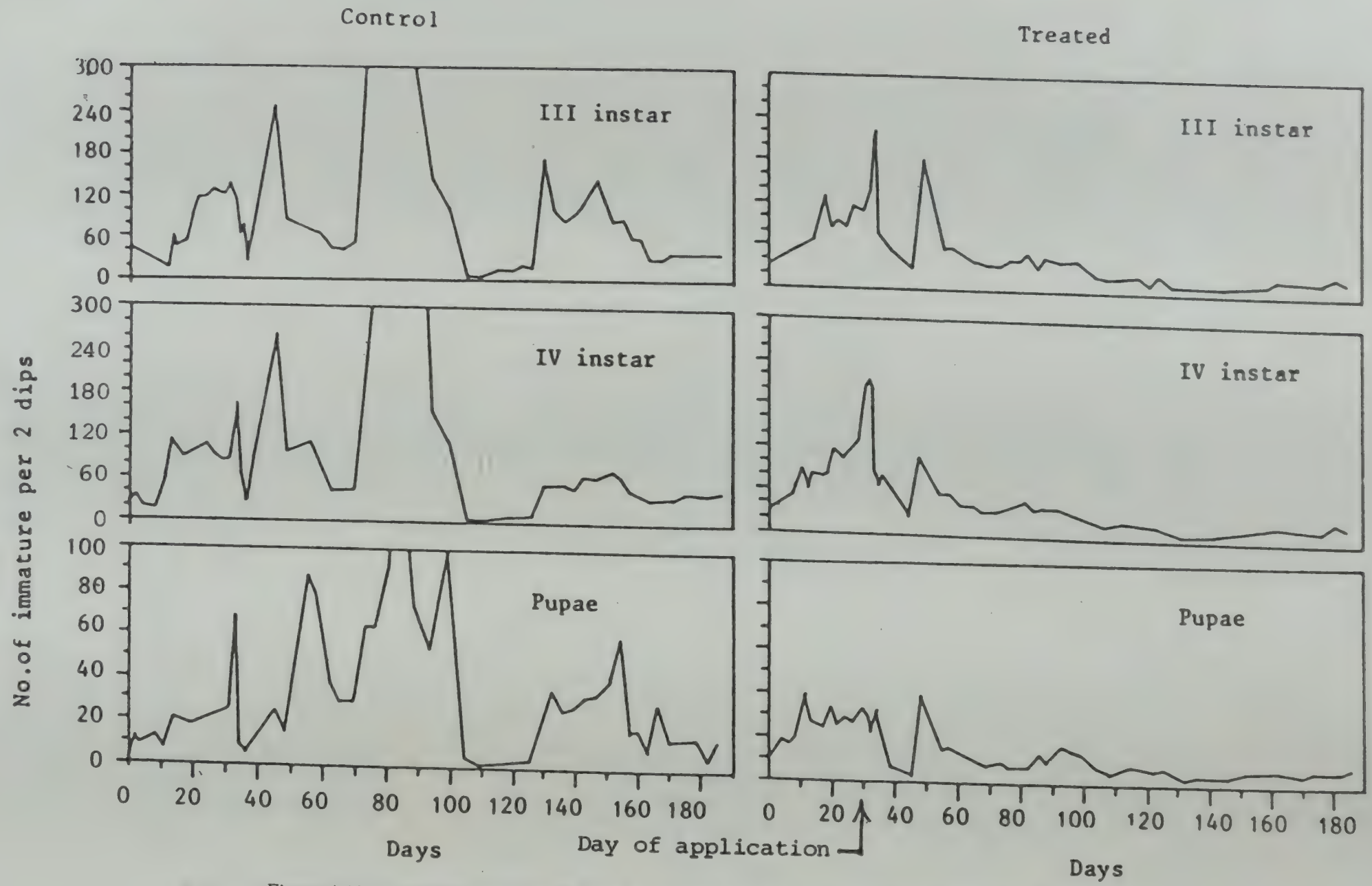


Fig *Figure* 4.10. Larvicidal activity of Deltafix, a slow release formulation of B.t H 14 on immatures of *Culex quinquefasciatus* breeding in wells.

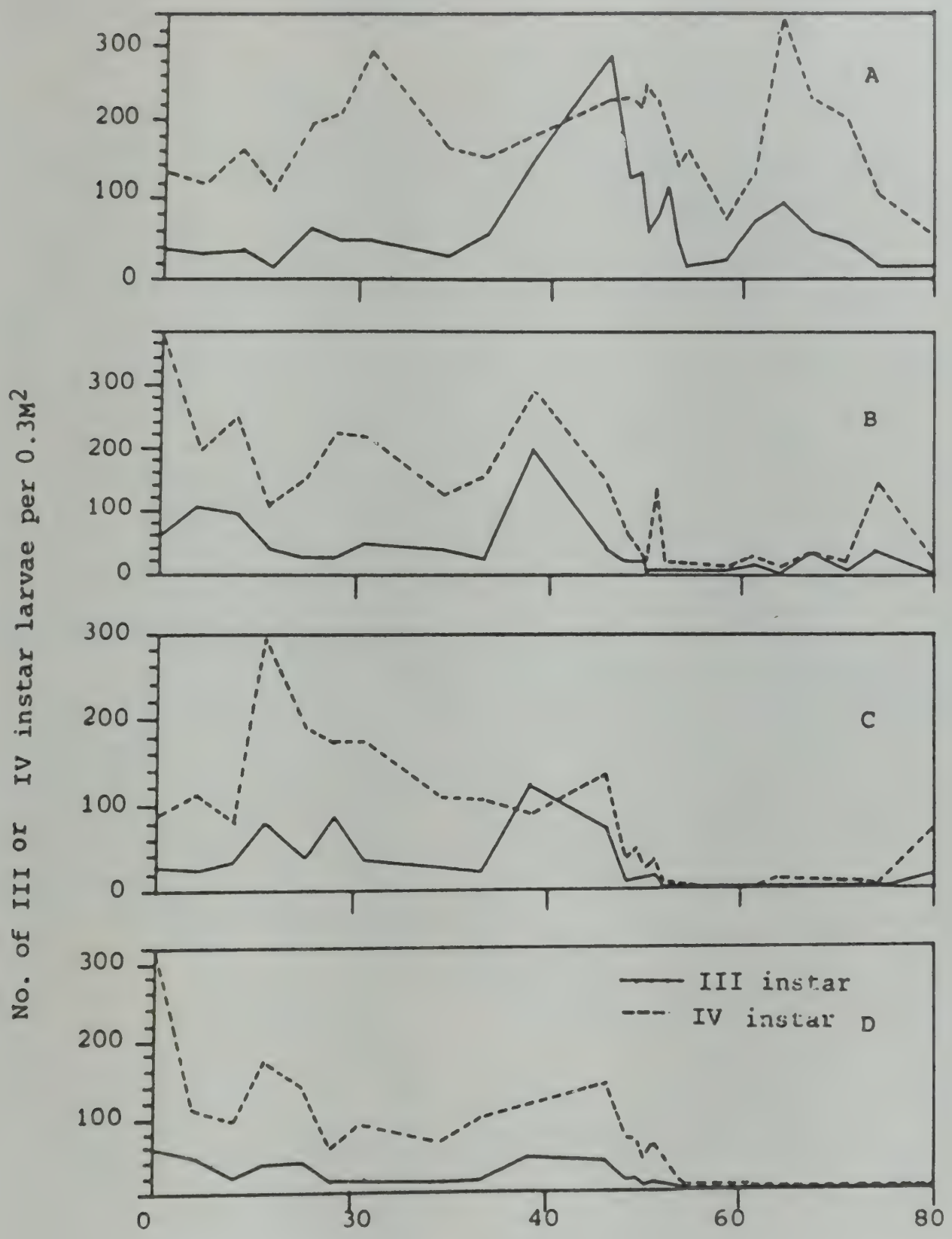


Figure 4.11. Effect of SPHERIFIX, a Slow Release Formulation of *Bacillus sphaericus* B42 on *Mansonia* sp. breeding in ponds (Control: A, Treated: B-5 kg/h, C-10 kg/h and D-15 kg/h).

The larvicidal activity of the mutants was determined and compared with that of the wild type strains, VCRC B42, 2362 and 1593. They were tested against *Culex quinquefasciatus*, *C. tritaeniorhynchus*, *Anopheles culicifacies* and *A. stephensi*. The Lc50 values obtained were compared with that of B42 and potency ratios (PRs) were calculated keeping the potency of B42 as 1.0 and the values are given in the Fig. 4.13. The Lc50 values of B42 were 80,160,720 and 4240 ng/ml respectively for *C. tritaeniorhynchus*, *C. quinquefasciatus*, *A. stephensi* and *A. culicifacies*. When tested on *C. tritaeniorhynchus*, except the mutants M12 and M13, all the rest were less toxic than the wild type strain. When tested on *C. quinquefasciatus* and *A. culicifacies* the M20 was found more toxic than the wild type strain, with potency ratios of 1.667 and 1.51 respectively, whereas the other mutants were less toxic. Against *A. stephensi* almost all the mutants were less toxic than the wild type strain. When the activity of the wild type strains 1593 and 2362 were compared with that of B42 against *C. tritaeniorhynchus*, *C. quinquefasciatus*, and *A. stephensi* they were found to be less toxic than the latter, with the only exception of 2362 showing higher activity against *A. culicifacies*; the potency ratio being 1.98. The results show that one of the mutants viz, M20 shows higher larvicidal activity than the wild type strain and lead to conclude that it should be possible to obtain more number of such improved strains through mutagenesis.

4.10. Fate of Bacillus thuringiensis H14 and Bacillus sphaericus applied to vector breeding sites.

Bacillus thuringiensis H14 and *B. sphaericus* have proved to be highly potent in controlling mosquitoes. There have been a few reports that *B. sphaericus* recycles in the treated sites with a few introductions. It was observed that if the spore count of *B. sphaericus* is more than 100 per ml it brought about reduction in the larval population. However, later reports state that these many number of spores were not encountered in the water beyond 150 days after treatment and therefore no larvicidal activity was noticed. In the case of *B. thuringiensis* H14, field experiments have indicated that its spore-crystal-complex does not stay in the larval feeding zone in adequate numbers to kill mosquito larvae beyond 24–48 h. The present investigation was taken up to understand the population dynamics of these two larvicidal bacilli when treated to mosquito breeding habitats, and the results are presented.

Two marker strains were used in this study. (i) a mutant of *B. thuringiensis* H14, MB24 which produces a dark brown pigment and (ii) a mutant of *B. sphaericus*, MS3, whose cells are macrofibrous and quite distinguishable from that of the wild type strain. The freeze-dried powders of the two bacterial agents were applied in diverse habitats, viz., rain water pools, casuarina garden pits, paddy fields and cess pits. The rain water pool was apparently unpolluted. The casuarina garden pit water, though was clear, had decaying vegetation at the bottom. The paddy fields had clear water and the cess pits contained more organic pollutants. While the former three habitats harboured *Anopheles* and *Culex vishnui* gr. larvae the cess pits had *Culex quinquefasciatus* larvae. The larvicides were suspended in water and applied to the breeding sites with Knapsack sprayers at the doses of 20 mg/M^2 (MB24) and 50 mg/M^2 (MS3).

The population of larvae present in the treated sites were monitored on different days by random sampling. The samples collected from each site were pooled, larvae and pupae were counted and the data was recorded as average numbers of early or late instar larvae or pupae per dip. The immatures were released back into the sites after counting.

The population of MB24 and MS3 in the treated sites were monitored on different days. Two samples each of soil and water from a habitat, were taken randomly, pooled, serially diluted and inoculated to appropriate agar surfaces. The agar plates were incubated for 48th at 30 $\pm$ 2°C and the colony forming units (CFU) were counted and recorded. The marker traits of the two mutants viz. dark brown pigment of MB24 and rough colony morphology and the macrofibre cell structure of MS3 were used to differentiate the mutants from the wild types, if any, and other bacteria.

Bacillus thuringiensis H14 (MB24): In the rain water pool, 50 $\times$ 10^3 CFUs/ml of water were found 1 h after treatment (Fig: 4.14). A day later there were 44 $\times$ 10^3 CFUs/ml and on 4th day the count decreased to 4 $\times$ 10^3/ml. On 7th day there

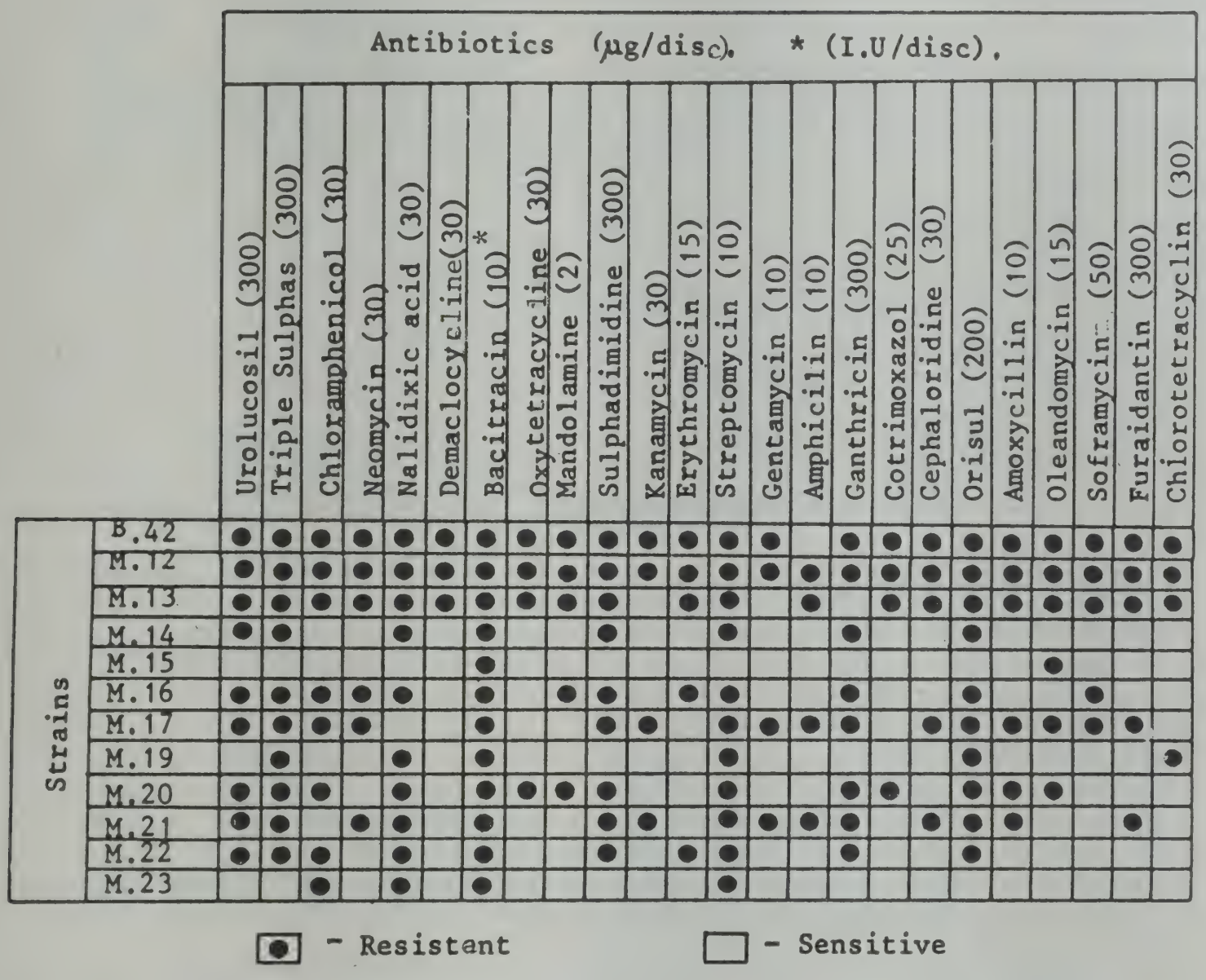


Figure 4.12. Antibiotic sensitivity / Resistance pattern of *Bacillus Sphaericus* B. 42 and its mutants.

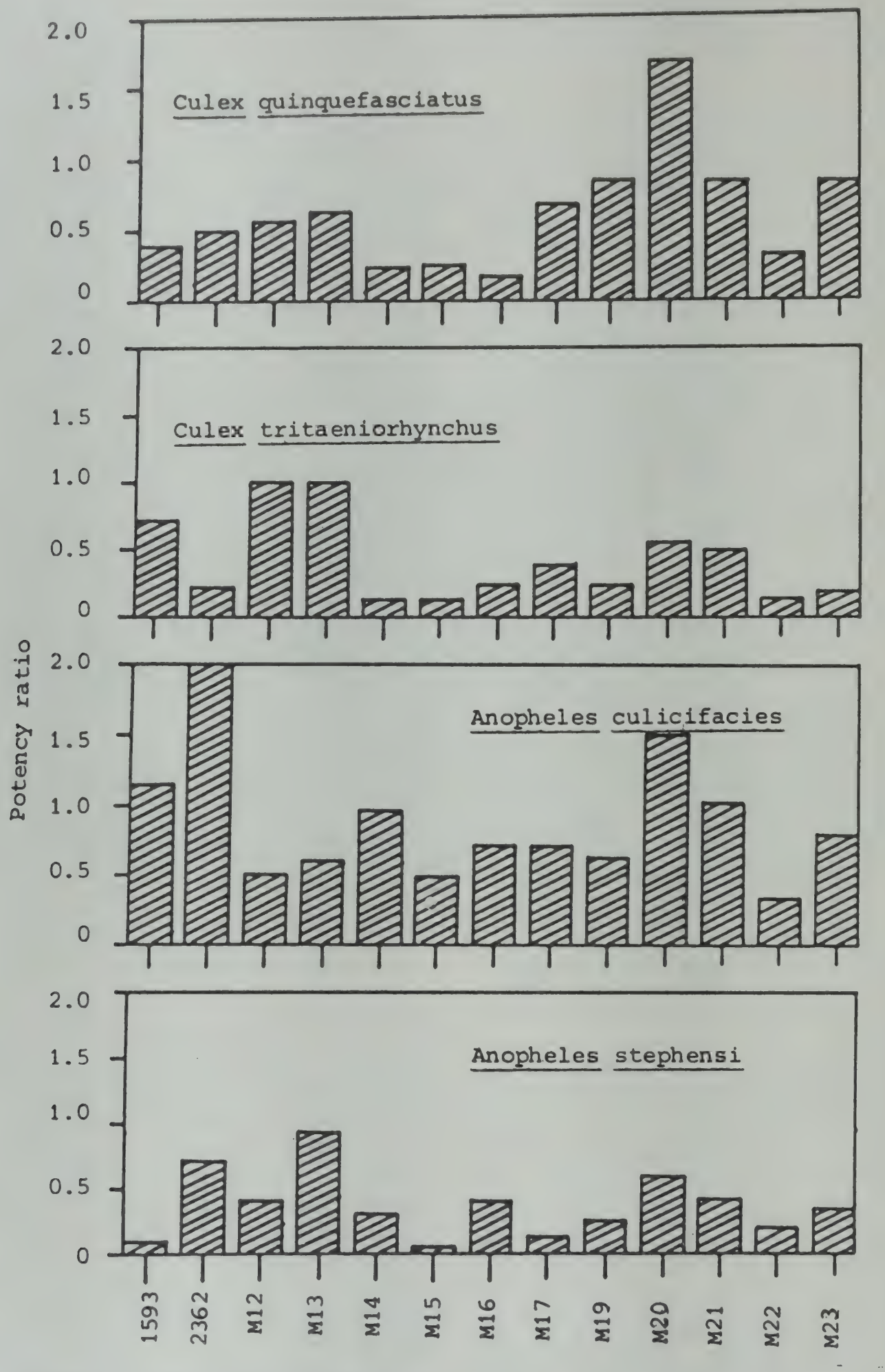


Figure 4.13. Potency ratios of *Bacillus sphaericus* mutants and of strains 1593 and 2362 against mosquito larvae when potency of strain B42 was used as basis.

were only 2 × 10^3 CFUs/ml which remained more or less constant till 35th day. The soil sample collected 1 h after treatment had 230 × 10^3 CFUs/g and 1 day later this number increased to 438 × 10^3/g. The samples collected on the 4th day contained 3400 × 10^3 CFUs/g which rose to 4000 × 10^3 by 7th day. From 7th to 19th day the count remained, more or less constant, but by 28th day it declined to 3 × 10^3/g. It was not possible to collect samples from this habitat beyond 35th day due to disturbance of the site. The results show that after 24 h of treatment there is a steep decline of *B. thuringiensis* H14 count from the larval feeding zone, with concomitant, increase in the soil samples, indicating that the cells disappearing from the water column had settled down in the bottom sediments. The increase in the number of *B. thuringiensis* H14 cells in the soil is obviously due to the addition of the cells from the water column and not due to multiplication of the bacterium in the habitat, because the cell count remains constant from 4th to 19th day and then drastically goes down.

In the water samples collected from casuarina garden pits, 45 × 10^3 CFUs/ml were detected within 1 h after treatment (Fig. 4.14). One day later there were only 4 × 10^3 CFUs/ml which further declined to 1.5 × 10^3/ml by 4th day. On 7th day there were only 7 × 10^2 CFUs/ml which gradually declined to 15 CFUs/ml by 21st day. Thereafter only a few numbers were detected upto 203rd day. In the soil sample 85 × 10^3 CFUs/g were detected 1 h after treatment which increased to 200 × 10^3 CFUs/g by 24th h.

Thereafter the population declined from 4th to 21st day, and the soil samples contained less number of CFUs which fluctuated between 16 × 10^3. to 64 × 10^3. From 21st day onwards there was a sharp fall in the *B. thuringiensis* H 14 population and no CFUs could be recovered beyond 203rd day.

In cess pit water 94 × 10^3 CFUs/ml were present 1 h after treatment and the number decreased to 51 × 10^3 24 h later (Fig. 4.14). On the 4th day there were only 18 × 10^3 CFUs/ml and thereafter there was a rapid decrease and on 28th day there were only 7 × 10^2 CFUs/ml. Between 28th day to 203rd day no cells could be detected in the larval feeding zone. In the soil samples there were 50 × 10^3 CFUs/g, 1 h post treatment. Samples collected on 1st and 4th day contained 58 × 10^3 and 55 × 10^3 CFUs/g respectively. From 7th to 21st day post-treatment there was a steady increase in the count *i.e.* 140 × 19^3 to 700 × 10^3/g. On 28th day, 78 × 10^3 CFUs were recovered per g of soil and thereafter the number decreased and on 203rd day there were only 2 × 10^3 CFUs/g.

In paddy field water 19 × 10^3 CFUs/ml were detected 1 h after treating *B. thuringiensis* H14 which decreased to 10 × 10^3 24 h later (Fig. 4.14). On 4th day there were only 6 × 10^3 CFUs/ml which decreased to 1 × 10^3 by 21st day. Thereafter, the count decreased rapidly and on 203rd day only 26 CFUs/ml were present. In the soil samples 500 × 10^3 CFUs/ml were found 1 h after application and this count lowered to 80 × 10^3 CFUs/g and 49 × 10^3 CFUs/g on 1st and 4th days, respectively. From 7th to 181st day the *B. thuringiensis* H14 count fluctuated between 10^3 – 10^4/g and beyond 203rd day no CFU could be recovered.

The results show that considerable numbers of *B. thuringiensis* H14 spores settled down from the water surface within a short span of 1 h post-treatment. In most of the habitats where it was introduced the number of CFUs/g of soil is higher than in 1 ml of water within 24 h after treatment, there is a sharp fall in the *B. thuringiensis* H14 count in the water samples collected with attendent increase in the count in the soil. From 1st day onwards there is steady decline of *B. thuringiensis* H14 population in the water until 240 days in cess pit and casuarina garden pit. However, in paddy field water the count continues to be more or less constant from 1–28 days and thereafter it declines gradually to 0.

The *B. thuringiensis* H14 count in the soil sample of cess pit and casuarina garden pits is quite high until 28 days and thereafter drops down slowly and no *B. thuringiensis* CFUs were found beyond 240 days. In the paddy field soil *B. thuringiensis* H14 population, wide fluctuations were observed on different days probably due to shallowness of the water column, but even here no CFUs could be found after 240 days.

Bacillus sphaericus: In rain water pools 27 × 10^3 *B. sphaericus* CFUs were detected 1 h post-

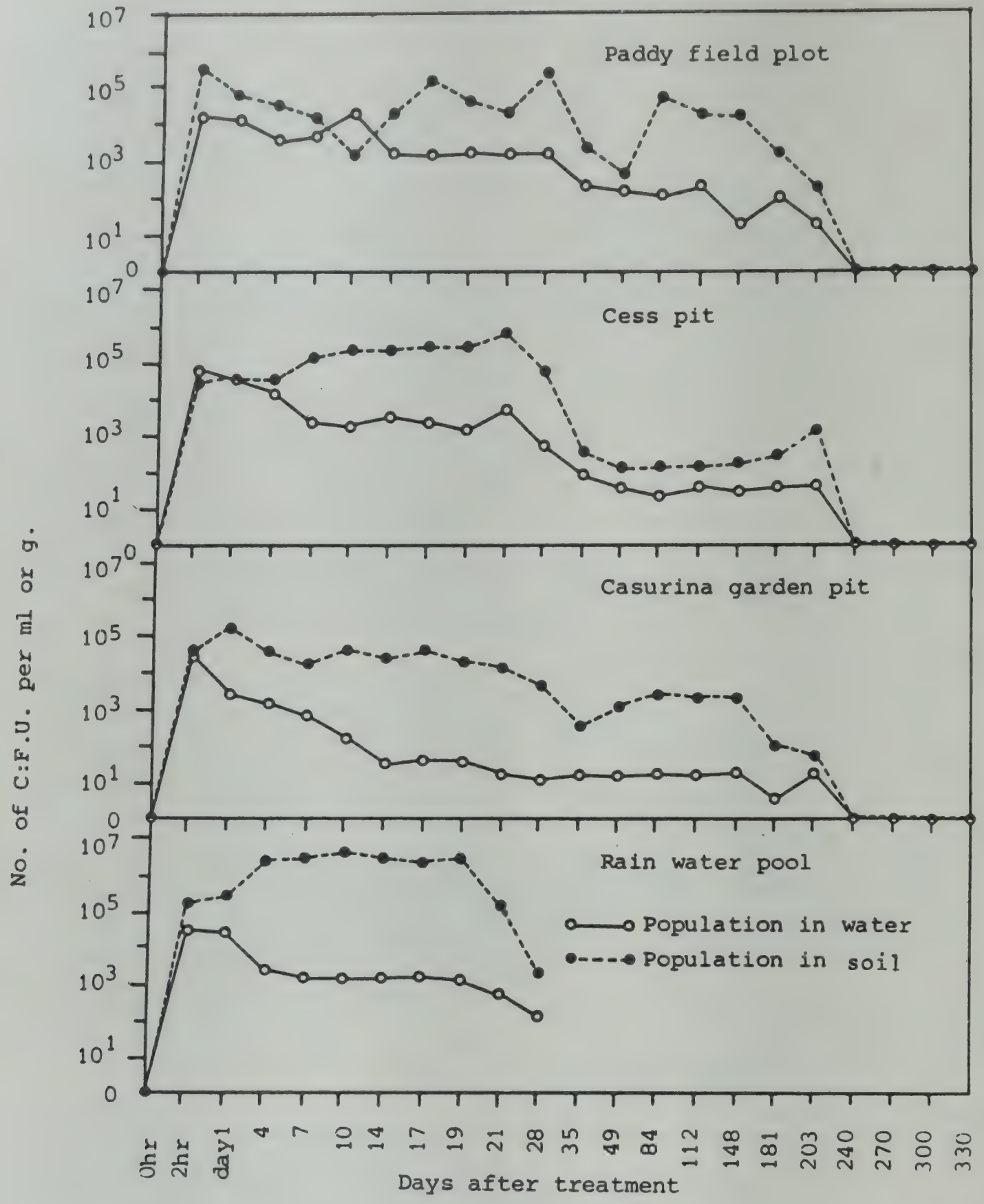


Figure 4.14. Population Dynamics of *Bacillus thuringiensis* H14 (MB 24) in the treated habitats.

treatment and from 1st to 10th day this number fluctuated between 2×10^3 and 14×10^3 CFUs/ml (Fig. 4.15). From 14th–19th day post-treatment $7–8 \times 10^2$ cells/ml were found and on 28th day only 52 cells/ml were detected. Soil samples collected from this habitat 1 h post-treatment contained 171×10^3/g by 24 h. Thereafter, the number of CFUs declined gradually upto 19th day post-treatment when 18×10^3 CFUs were recovered. On 28th day there were only 4×10^2 CFUs.

In casuarina garden pit 43×10^3 CFUs/ml of water were detected 1 h after application and by 24th h this number lowered to 5×10^3/ml (Fig.4. 15). From 4th day to 17th day the *B. sphaericus* number in the larval feeding zone declined gradually. On 17th day there were only 2×10^2 CFUs/ml and beyond 19th day no *B. sphaericus* CFUs were detected. In the casuarina garden pit soil 23×10^3 CFUs/g were recovered 1 h post-treatment which increased to 31×10^3 by 24th h. Thereafter, the number obtained per gm of soil declined gradually and on 270th day 2×10^2 CFUs were recovered.

One hour after aplication of *B. sphaericus* the cess pit water contained 36×10^3 cells/ml and by 24th h there were 48×10^3 cells (Fig. 4.15). On 4th and 7th days there were 28×10^3 and 35×10^3 CFUs/ml. Thereafter, the *B. sphaericus* cells disappeared slowly from the water till 19th day when only 1×10^3 CFUs/ml were recovered. The cesspit soil sample taken 1 h after treatment of *B. sphaericus* contained 150×10^3 CFUs/g which remained more or less constant till 19th day. Thereafter, and till 84th day the number fluctuated between $10^2–10^4$ cells/g. Further samplings showed a rapid decline in the number and on 30th day there were only 25 CFUs/g.

The paddy field water sampled after 1 h of treatment with *B. sphaericus* contained 43×10^3 CFUs/ml which decreased to 16×10^3 by 24th h (Fig. 4.15). From 4th–49th day the *B. sphaericus* count fluctuated between 10^2 and 10^3 Thereafter, the number declined rapidly and on 140th day it was 35/ml (fig. 4.16). No CFUs were detected in the water beyond 140th day. The paddy field soil collected 1 h after treatment contained 1000×10^3 CFUs/g and by 24th h this number declined to 950×10^3. From 4th to 17th day the *B. sphaericus* number fluctuated between $133 \times 10^3 – 230 \times 10^3$ CFUs/g. Beyond this period there was a rapid decline in the *B. sphaericus* count but it could be detected in the soil upto 270th day.

The data show that significant numbers of *B. sphaericus* spores settle down to the bottom soil of treated sites within 1 h of application. They totally disappear from the waters of cesspits and casuarina garden pits after 17–19 days of treatment But in paddy field water, they were found upto 148 days. This may be due to refloating of those settled down in the soil due to frequent disturbances of the shallow water column. In the soils, the *B. sphaericus* continued to survive upto 270–300 days, but in low numbers.

4.11. Studies on the antagonistic microorganisms of Bacillus thuringiensis H14 and Bacillus sphaericus

Investigations were undertaken to look for the likely antagonists of *Bacillus sphaericus* and *B. thuringiensis*–H14 in 3 habitats where mosquitoes breed prolifically. They are, cesspits, casuarina garden pits and paddy fields. From each habitat 5 samples were collected, pooled and plated on appropriate media to isolate bacteria, fungi and actinomycetes. From cesspits, 51 bacteria, 31 fungi, and 29 actinomycetes, from casuarina garden pits, 39 bacteria, 41 fungi and 45 actinomycetes, and from paddy fields 39 bacteria, 22 fungi and 42 actinomycetes were obtained.

Of the 39 bacterial isolates from casuarina garden pits, 5 inhibited *Bacillus sphaericus* and the diameter of inhibition zone was in the range of 10–17 mm. Among the 30 bacterial isolates from paddy field 4 inhibited *B. sphaericus* and the zone of inhibition ranged from 11 mm to 14 mm. Six isolates out of 51 from cess pits were inhibitory to *B. sphaericus*. Among these bacterial antagonists 9 are gram + ve, 6 are gram – ve and 2 are cocci. Further studies are in progress.

4.12. Studies on the metabolites of the fungus, tolypocladium

That one of the fungal isolates produces larvicidal factors as well as an antibiotic was reported earlier. This fungus was identified as *Tolypocladium*, belonging to deuteromycetous

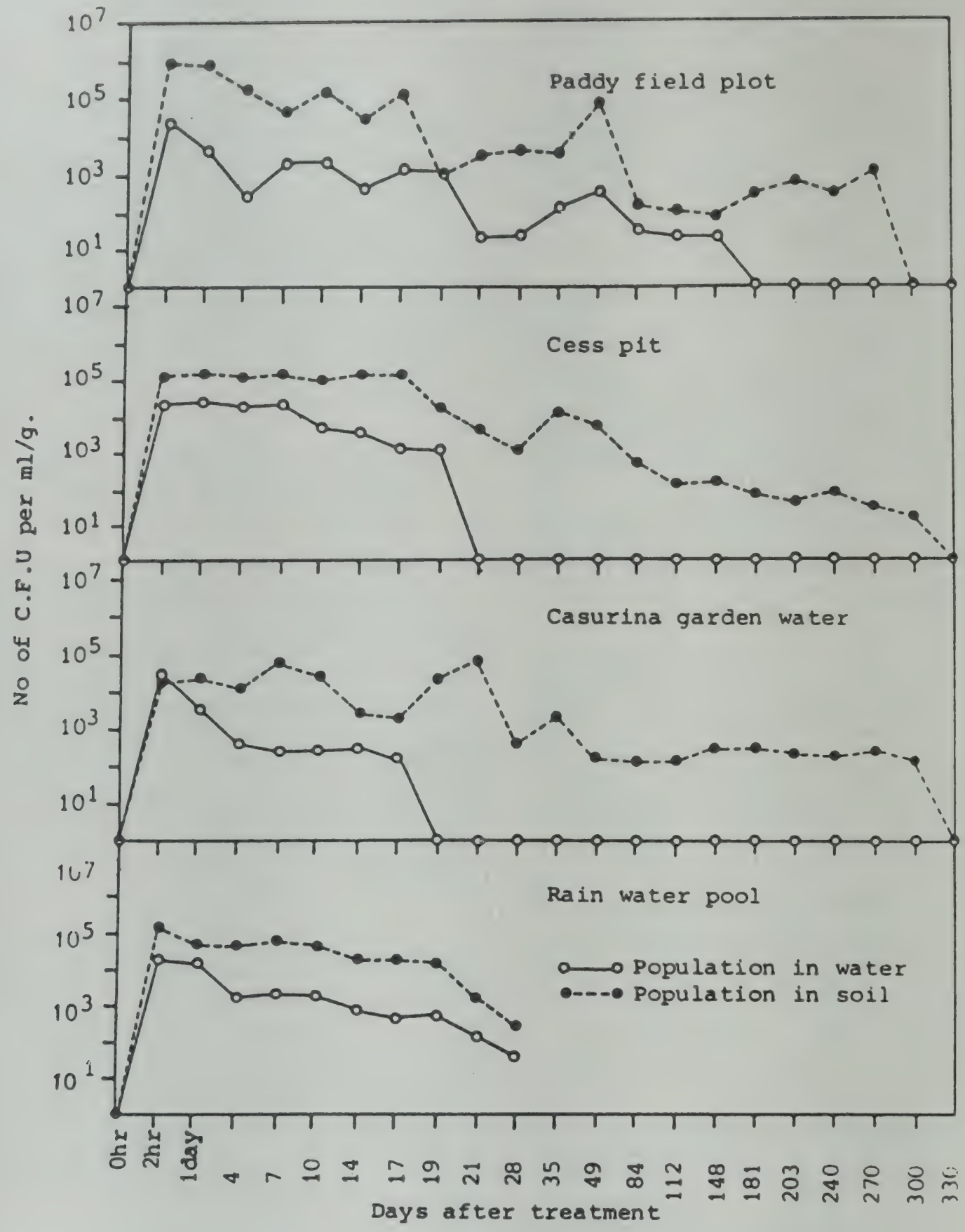


Figure 4.15. Population Dynamics of *Bacillus sphaericus* (MS 3) in the treated habitats.

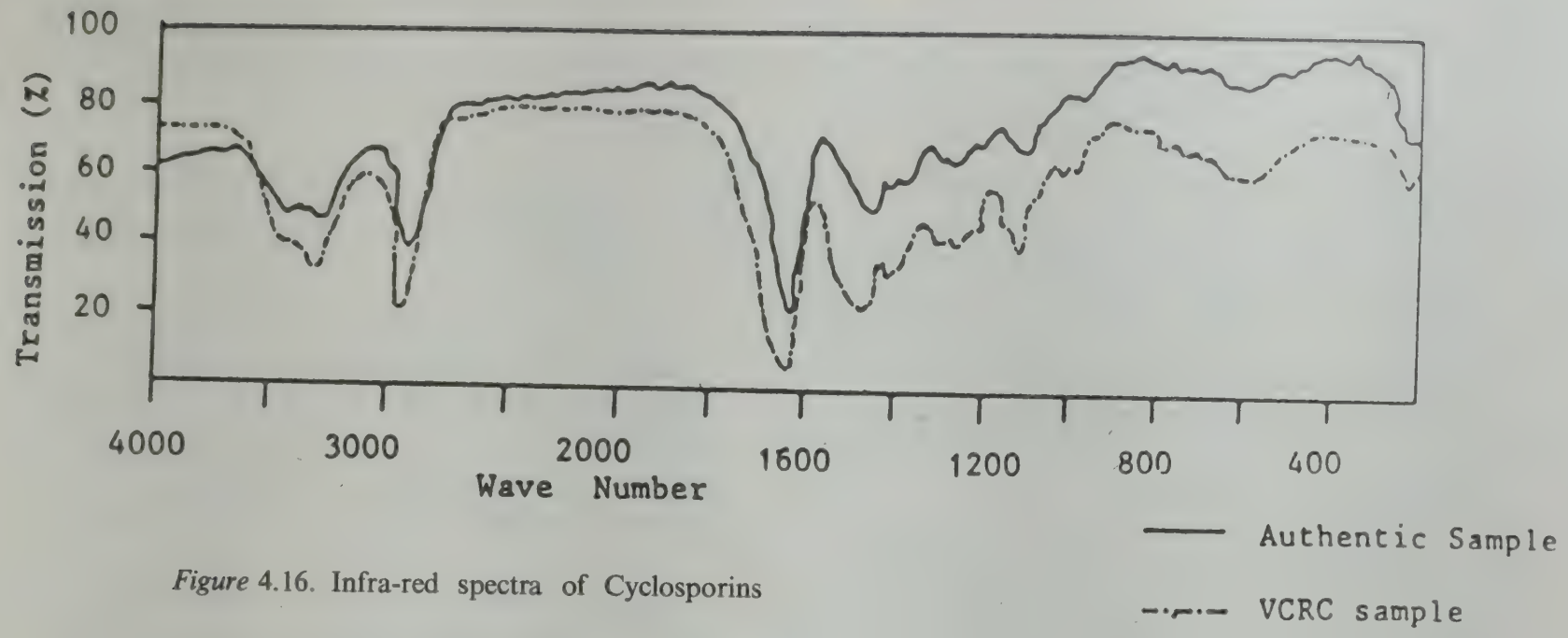


Figure 4.16. Infra-red spectra of Cyclosporins

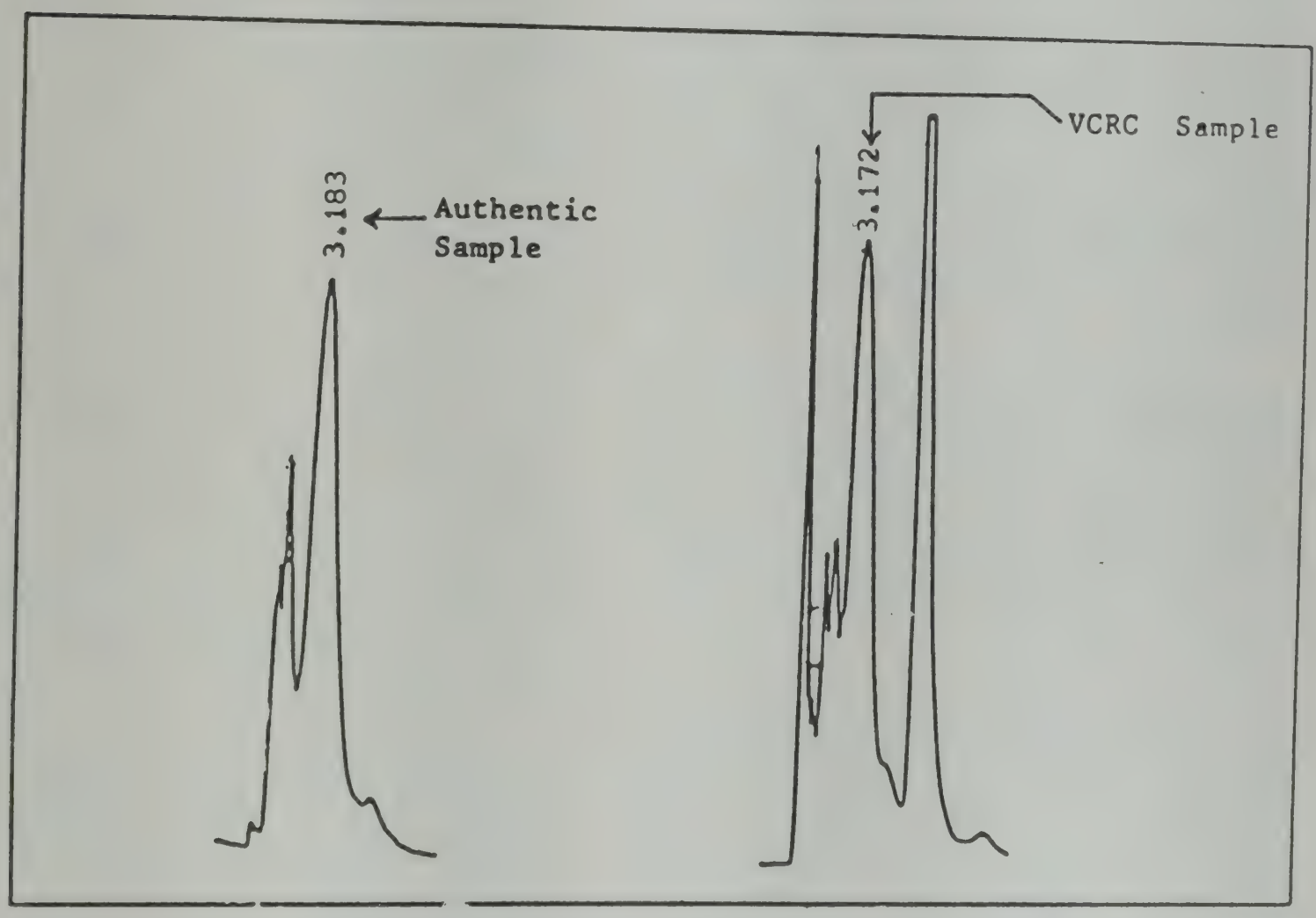


Figure 4.17. HPLC Profiles of Cyclosporins

group. This fungus grows very well in simple media at room temperature. It utilizes carbohydrates like glucose, maltose, lactose, corn starch, cassava starch and polyhydric alcohols. Better growth occurs in the presence of casamino acids. The fungus attains maximum growth in about three weeks time and the biomass yield varies from 30 g/l to 50 g/l. The fungal biomass was extracted with ethyl acetate and the extract was fractionated by column chromatography. The fractions were read for absorbance at 214 nm and those showing maximum absorption were pooled, further purified and used for analyses.

The purified fraction was subjected to IR and NMR spectral studies and the spectra obtained were superimposable on to the spectra obtained with pure cyclosporin (Fig. 4.16). HPLC was done with methanol-water as mobile phase for comparison of the VCRC sample with authentic sample. The HPLC profile of the sample and the standard were identical in relation to the retention time (Fig. 4.17). The absorbance ratio of the test sample was compared with that of authentic cyclosporin. Both the test sample and cyclosporin gave an absorbance ratio of 0.49 at 205 nm and 210 nm. The ratio also matched at 210 nm and 225 nm.

A suitable dilution of the test sample was made in normal saline and checked for immunological relationship by RIA. The following crude samples were also tested similarly: i. static culture extract, ii. shake culture extract, iii. culture supernatant and iv. fermentor culture extract. From the results it is concluded that all the samples listed above resemble cyclosporin in their immunochemical reactivity.

To study the *invivo* effects of the test sample Delayed Type Hypersensitivity (DTH) and Haemagglutination titre (HA) were measured using sheep RBCs (SRBC) as antigens in adult mice. As the DTH response measured in the animals receiving the authentic cyclosporin was not different from that of the control animals it was not possible to compare the effect of the test sample. The antibody titre to SRBC, an indicator of the effect on humoral immunity, was significantly decreased in the animals dosed with the test sample which was comparable to that of cyclosporin treated animals.

Further studies are in progress to optimise culture conditions so as to obtain maximum yield of the fungal metabolite and to simplify downstream processing.

4.13. Production of melanin and L-Dihydroxy phenylalanine by a Bacillus thuringiensis H14 mutant

At the Vector Control Research Centre a mutant of *B. thuringiensis* H14 (BtH14) was developed which produces not only the larvicidal factor but also an extracellular dark brown pigment. Further studies were carried out on this pigment and the results are presented here.

The pigment produced by the BtH14 mutant was secreted into the culture broth and also deposited on the cells. All the tests carried out to identify the pigment were positive for melanin. Its IR spectrum was similar to those reported for standard melanin (Fig. 4.18) showing absorbances at 2.9 um to (–OH), 3.5 um to (–CH), 5.9 um to carboxylic or phenolic groups and 6.1 um to H-bonded quinone or unsaturated groups. The incorporation of the amino acid, tyrosine in the growth medium induced the production of higher quantity of melanin followed by the media incorporated with phenylalanine and cysteine. The results indicate that tyrosine acts as the precursor of melanin. The incorporation of sodium ascorbate or L-arginine in the growth medium inhibited the formation of melanin by the mutant while its absence lead to the normal synthesis. TLC analysis of the culture supernatant of the mutant grown in the presence of ascorbate showed the presence of a spot which co-migrated with that of standard L-3, 4 dihydroxy phenylalanine (DOPA). This was further confirmed by HPLC analysis (Fig. 4.19).

The investigation shows that the mosquitocidal BtH14 mutant produces melanin and it is possible to block the melanin synthesis pathway at L-DOPA leading to the accumulation of the latter in the culture broth. L-DOPA is a neurotransmitter precursor of dopamine and hence has wide applications for the treatment of hypertensive diseases. Because of its great demand search is on for less expensive methods of production of this drug. At present the main sources are plants and to some extent microorganisms. The BtH14 mutant used in the present study not only has the potential of producing L-DOPA but also has got

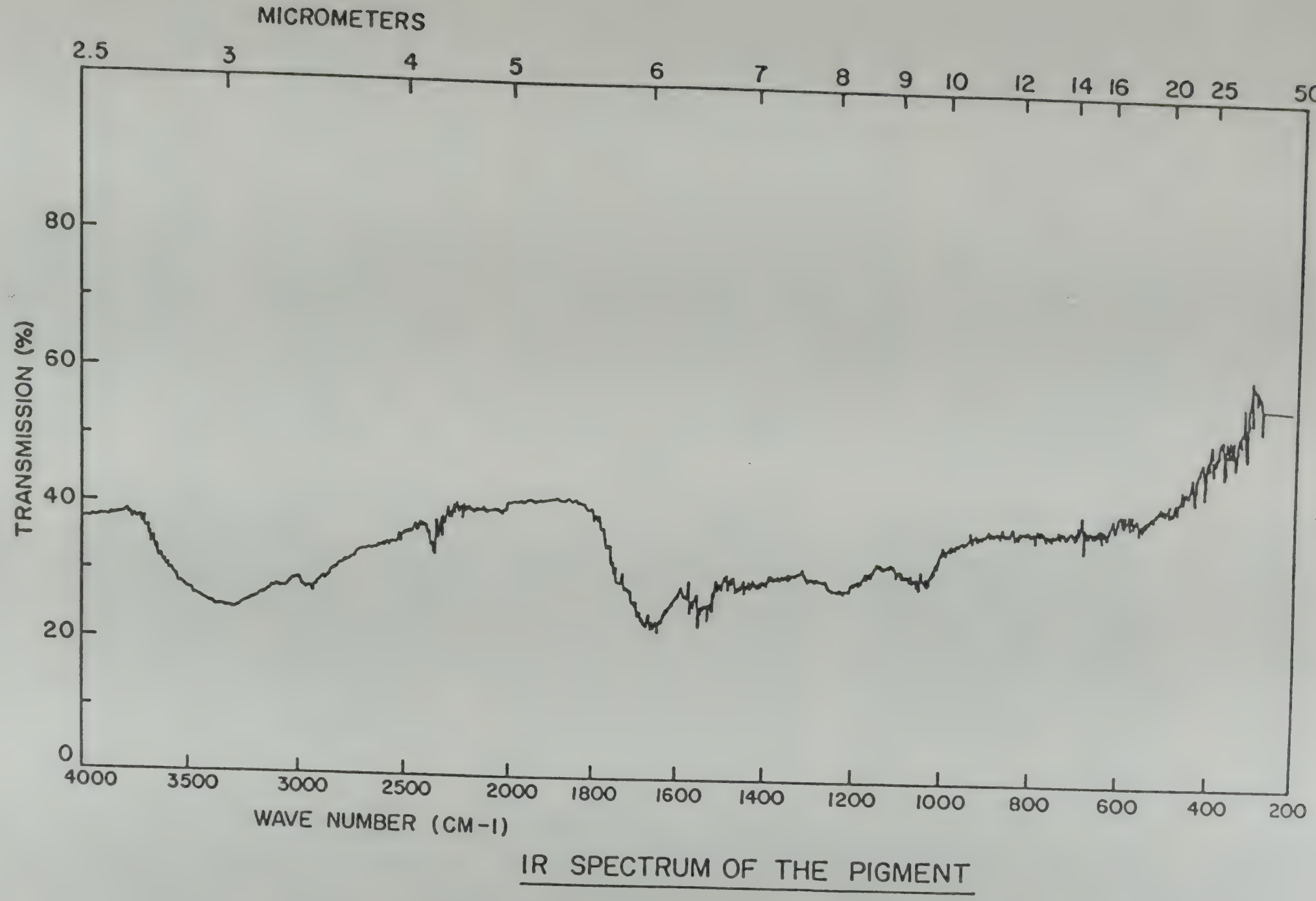


Figure 4.18. IR spectrum of the pigment of *Bacillus thuringiensis* H. 14 (MB 24).

high larvicidal activity. The added trait of L-DOPA production by the mutant in addition to the entomotoxin, makes it more attractive for commercial exploitation.

4.14. Romanomermis iyengari

4.14.1. Effect of host-parasite ratio and temperature on parasitism and development of Romanomermis iyengari

The efficiency of mass production of *R. iyengari* is dependent upon several factors such as the number of nematodes produced and the proportion of nematode differentiated into healthy females. Therefore, a study was undertaken to understand the effect of host-parasite ratio (HPR) and temperature on parasitism and sex differentiation of the nematode.

In this study *C. quinquefasciatus* larvae were exposed to preparasitic nematodes at different HPR and at 20°, 25°, 30° and 35°C. The mosquito larvae were reared at these temperatures giving equal amount of food until the emergence of nematodes. Observations were made for parasitism and the number and sex of post-parasites that emerged from each infected larva. The results (Fig. 4.20) showed that the percentage parasitism was high (86–92%) at 25°C and 30°C and at the HPR of 1:3 and 1:4 whereas at 20°C and 35°C and at the same HPR the paraitism was 70–82%. However, at the HPRs of 1:8 and 1:10, 84–94% parasitism was observed at these temperatures. Most of the parasites differentiated into females at 20°C and 25°C at all the host-parasite ratios (Fig. 4.21). At higher temperatures (30°C and 35°C), and high HPRs the tendency was to differentiate into more number of males; but at low HPR, more number of parasites differentiated into females. Invariably more number of parasites/mosquito larva emerged at 20°C and 25°C than at 30°C and 35°C (Fig. 4.22). Analysis of the data to understand the influence of parasite burden on the differentiation of sexes indicate that at lower parasite burden more number of females were obtained whereas when the parasite burden is increased more number of males were obtained (Fig. 4.23).

The data thus show that i. low HPR and 25–30°C favour higher percentage parasitism, ii. low HPR and 20–25°C favour emergence of more number of females, iii. high HPR and 20–25°C favour more number of parasites host and iv. low parasite burden favours the emergence of more number of females.

The duration of parasitic life of *R. iyengari* in *C. quinquefasciatus* larvae was studied at different temperatures by infecting II instar larvae. The nematode completed its parasitic life in 3.5–5 days at 35°C, 4.5–6 days at 30°C and 6–8 days at 25°C. The parasitic life was found prolonged to 15 to 17 days at 20°C. The data show that lower temperatures increased the parasitic life while higher temperatures shortened it.

4.14.2. Reproductive Potential and Embryonic Development of Romanomermis Iyengari

Information on the number of eggs laid by the female *R. iyengari* and the duration required for egg maturation will help to understand the multiplication rate of the nematode in the laboratory culture. Therefore studies were conducted to understand the egg laying potential of *R. iyengari*. Female *R. iyengari* emerged from *Cx. quinquefasciatus* larvae were used for this study. These nematodes started laying eggs after 11 days of emergence at 29 $\pm$ 1°C. A single female laid 35–1179 eggs over a period of 13 days, the mean number being 751 per female. Occurence of peak oviposition was observed during the first 4 days.

The embryonic development of *R. iyengari* was observed at 30 $\pm$ 2°C. The embryos reached two cell stage and multiple cell stage within 6 h and 18 h of egg laying, respectively. The cresent stage, the early coil stage and late coil stage were reached, respectively, after 34, 58 and 78 h. Thus the total duration of embryonic development was 78 h and the eggs were found hatching 4 days after egg laying.

4.14.3. Improved culture and storage techniques for Romanomermis iyengari

The VCRC has been attempting to culture *R. iyengari* in water rather than in sand bed. Studies on the effect of CO_2 on hatching of eggs showed that the CO_2 treatment at different concentrations did not affect the infectivity of preparasites as shown in the Table 4.3. It was observed that if the number of nematodes seeded in

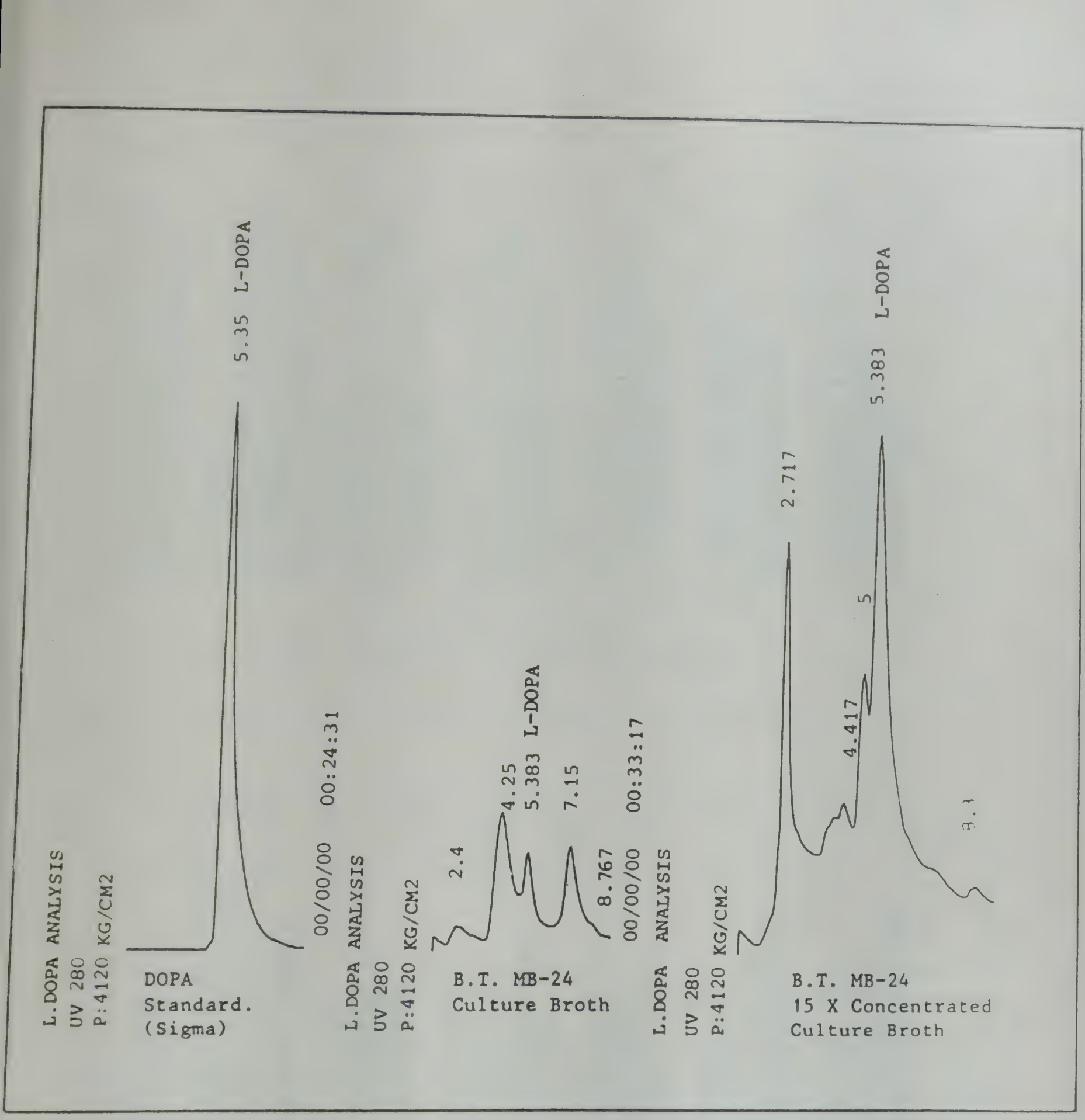


Figure 4.19. HPLC Analysis of DOPA from *Bacillus thuringiensis* H. 14 (MB-24) Culture Broth

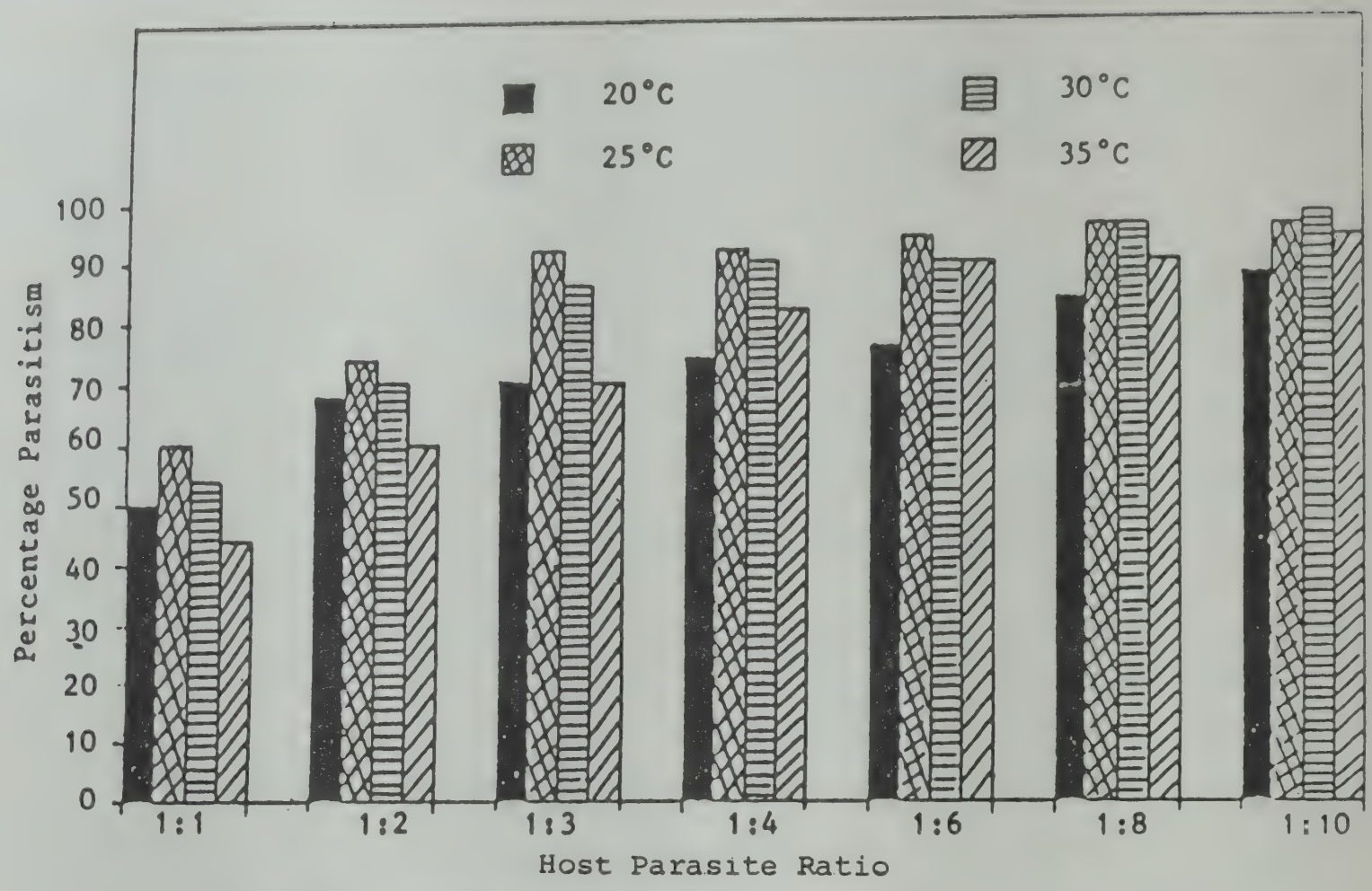


Figure 4.20. Percentage parasitism at different temperatures and hostparasite ratios

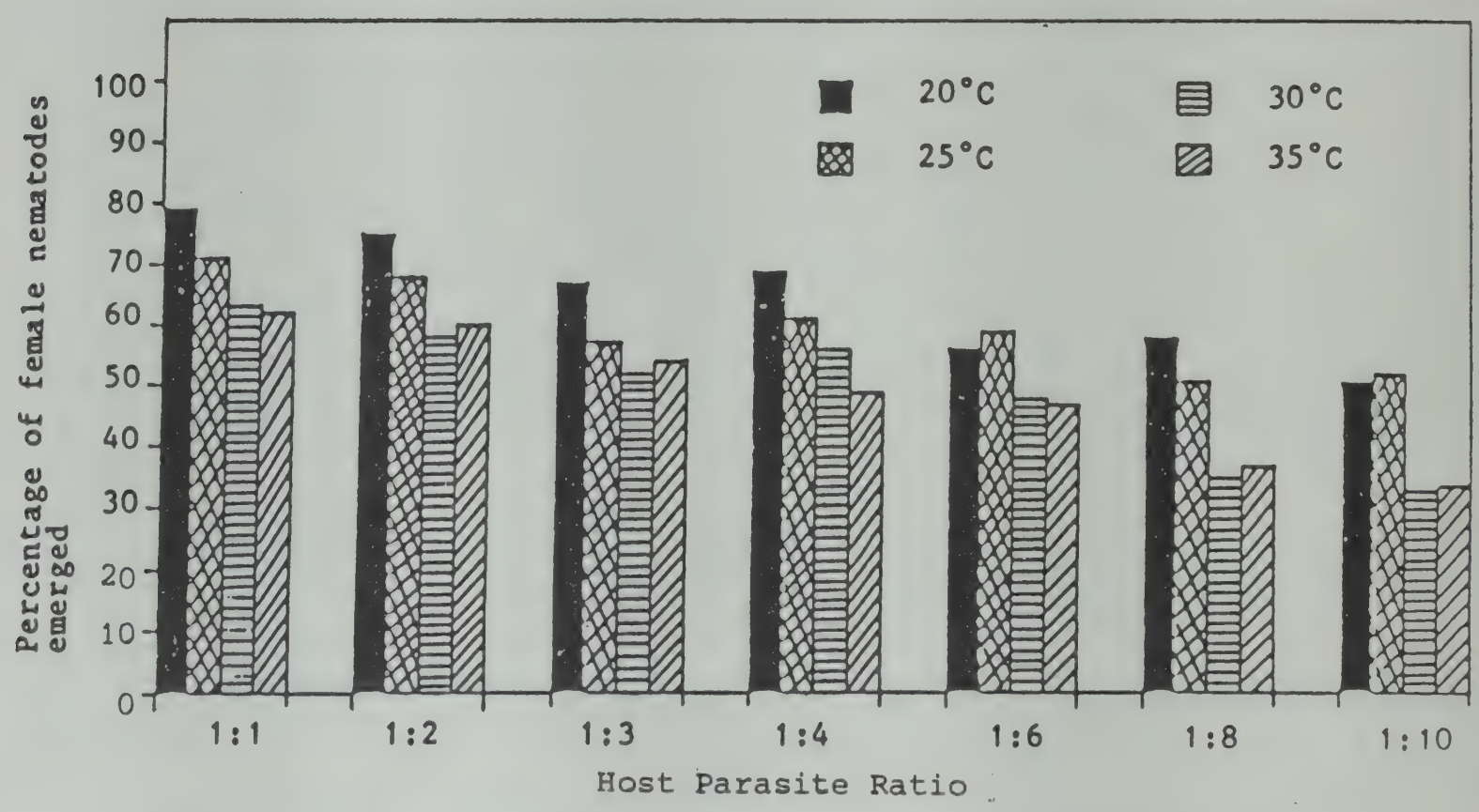


Figure 4.21. Percentage of female nematodes emerging at different temperatures and host parasite ratios.

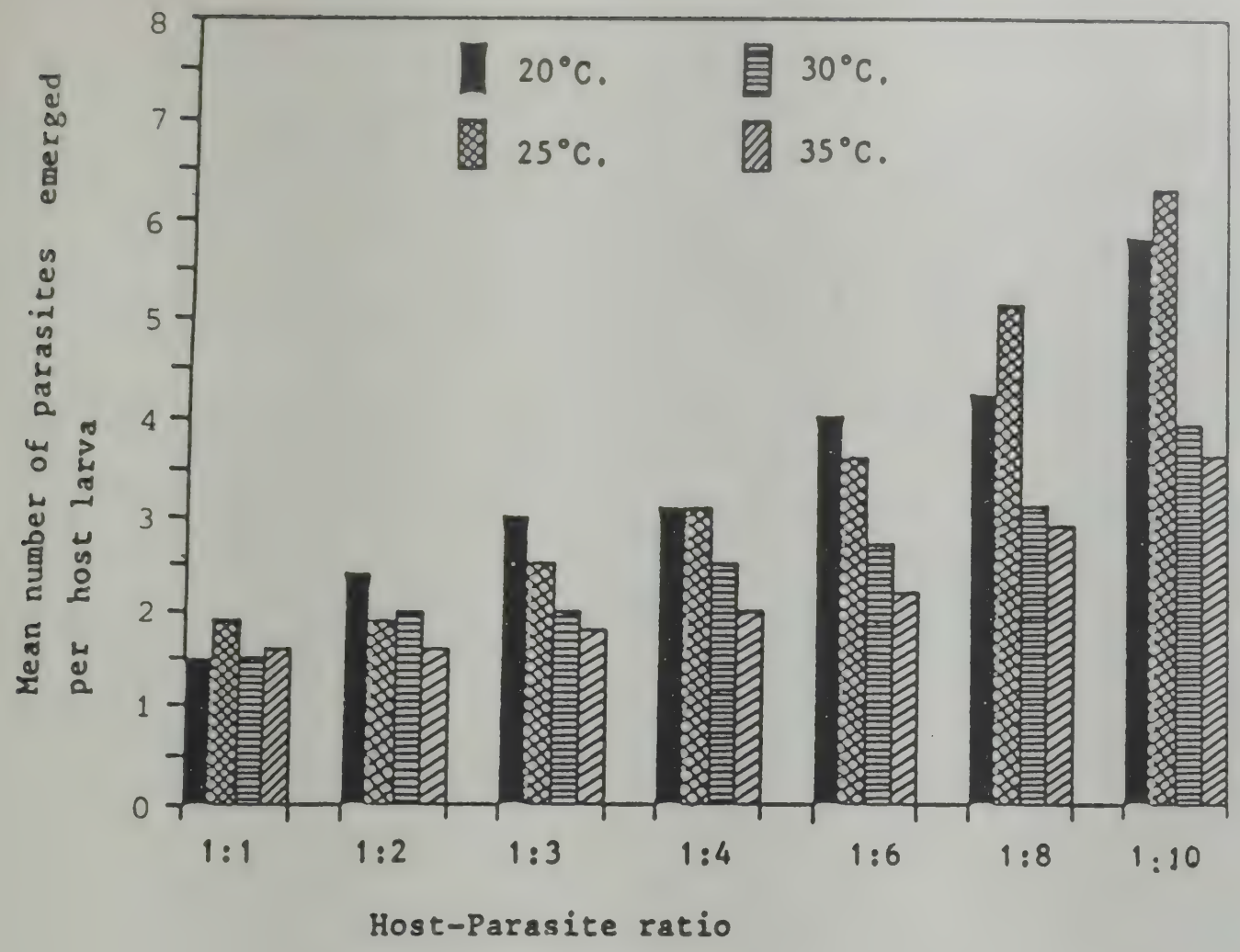


Figure 4.22. Mean number of Parasites per host larva at different temperatures and host-parasite ratios.

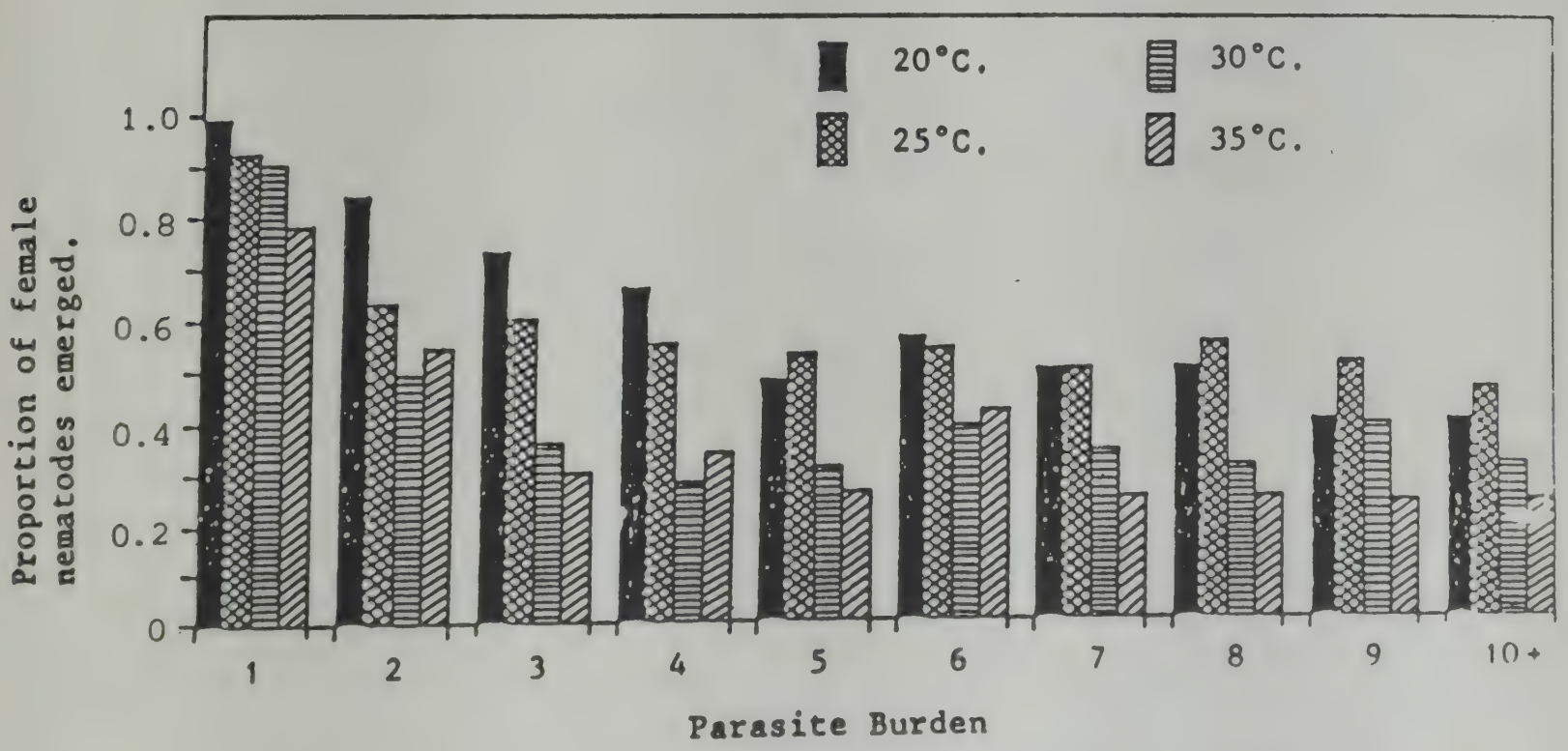


Fig *Figure* 4.23. Proportion of females produced at different temperatures and at different parasite burdens.

TABLE 4.3

Effect of CO_2 on the hatching of *Romanomermis iyengari* eggs

Tube Nos.	Conc. of CO_2 after treatment	Initial count of eggs/ml.	Percentage of eggs hatched after					% parasitism on *C. quinquefasciatus* II instar larvae
			1 hr	2 hrs	3 hrs	4 hrs	5 hrs	
1	15 ppm	104	13.5	30.8	57.7	67.3	73.1	92
2	30 ppm	96	20.8	31.3	52.1	72.9	87.5	98
3	60 ppm	108	24.1	33.3	55.6	75.9	83.3	95
4	120 ppm	86	73.2	39.5	65.1	79.1	88.4	96
5	180 ppm	90	33.3	44.4	82.2	88.9	97.8	90
6	240 ppm	100	30.0	36.0	76.0	92.0	96.0	100
7	300 ppm	90	31.1	46.7	62.2	82.2	88.9	98
8	360 ppm	110	18.2	43.6	72.7	87.3	90.9	100
9	Control in CO_2 free distilled water	104	0	4.3	5.8	9.6	11.5	100

water culture exceeds certain level, the egg production was delayed. An experiment was conducted to investigate this phenomena by seeding various numbers of male and female nematodes. The nematodes were seeded at 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 numbers/container at the male:female ratio of 1:1.

The moulting was delayed with increase in the numbers of the nematode seeded. About 79% of the nematodes moulted on the 6th day when the seeding rate was 100–400/container at 27–30°C. At the seeding rate of 500–800, 55% of them moulted and at 900–1000 only 7% of them moulted. For 100% moulting it took 8 days and 10 days at the seeding rates of 100–400 and 500–800 respectively, whereas only after 17 days 80% of the nematodes completed moulting in the case of 900–1000 seeding rate.

The results indicate that the nematodes at the seeding rate of 100–400 numbers/250ml would also produce more number of eggs. Studies on these parameters such as the egg production rate, the time required to complete egg laying etc. in varying numbers of seeding are in progress.

4.14.4. Developing pollution tolerant strains of Romanomermis iyengari

Two strains of *R. iyengari* capable of withstanding high pH and pollution were developed and reported earlier. These strains have completed several generations in sand cultures wherein cesspit water, with the following parameters is being used to keep the sand bed moist: pH:9.0; suspended solids:50mg/lit.; dissolved solids:4700 mg/lit.; conductivity:1600 micro mhos/cm.

One of the strains (obtained through EMS treatment) had poor reproductive potential, *i.e.*, producing low proportion of females (22%). Therefore this strain was again exposed to EMS to improve its reproductive potential and its traits are being studied. After repeated exposure of post-and pre-parasites to EMS for two generations, the production of females was found to increase from 22% to 36%.

Interestingly, from among the progenies of this mutant 2 intersex post-parasites out of 369 were noticed, which was not found in the parent strain so far. Another mutant, obtained through selection was studied for its tolerance to conductivity which is an important factor that decides the survival and infectivity of preparasitic nematodes in polluted water. This strain infects mosquito larvae (20% infection) at a conductivity of 3300 micromhos/cm in which the parent strain fails to do so. Further studies on the tolerance of this mutant to different chemical factors are in progress.

4.14.5. Field evaluation of Romanomermis iyengari in different mosquito breeding sites

R. iyengari was applied to grass lands, paddy fields, and irrigation tank, in Bangalore and the results were reported earlier. During the period under report more areas were brought under the field evaluation programme and various forms of the nematode were introduced in different mosquito breeding habitats. The observations recorded so far show that the nematode recycles in grass land irrigated with sewage water. Therefore the area of the trial plot was extended from 200 M^2 (last year) to 2000 M^2. The release of Pre-parasitic nematode (ppn) at a mean dosage of 5000 ppn/M^2 showed 74% parasitism initially among the different instars of larvae dissected. Recycling of the nematode was observed upto 114 days with varying levels of parasitism during the period (Fig: 4.24). About 4500 M^2 of paddy field was treated with pre-parasitic nematode at a dosage of 4000 ppn/M^2. This has resulted in 75% parasitism initially in different larval instars of *Cx. tritaeniorhynchus* and *Anopheles* sp. and the recycling of the nematode was observed on 22nd day causing 13.3% parasitism. This site dried up since then. About 200 M periphery of an irrigation tank was applied with post-parasitic nematodes at the dosage of 800/M. This has resulted in 12.5% parasitism 25 days after release. In this site the nematode got established and recycled for about 90 days.

Tree holes harbouring *Aedes albopictus* larvae were treated with pre-parasitic nematode at different host parasite ratio viz., 1:1 to 1:5. Parasitological evaluation showed 100% parasitism in different larval instars and the nematode was found recyling in this habitat with varying levels of parasitism upto 99th day (Fig: 4.25).

Water samples from all the treated sites were analysed for various parameters of pollution. The

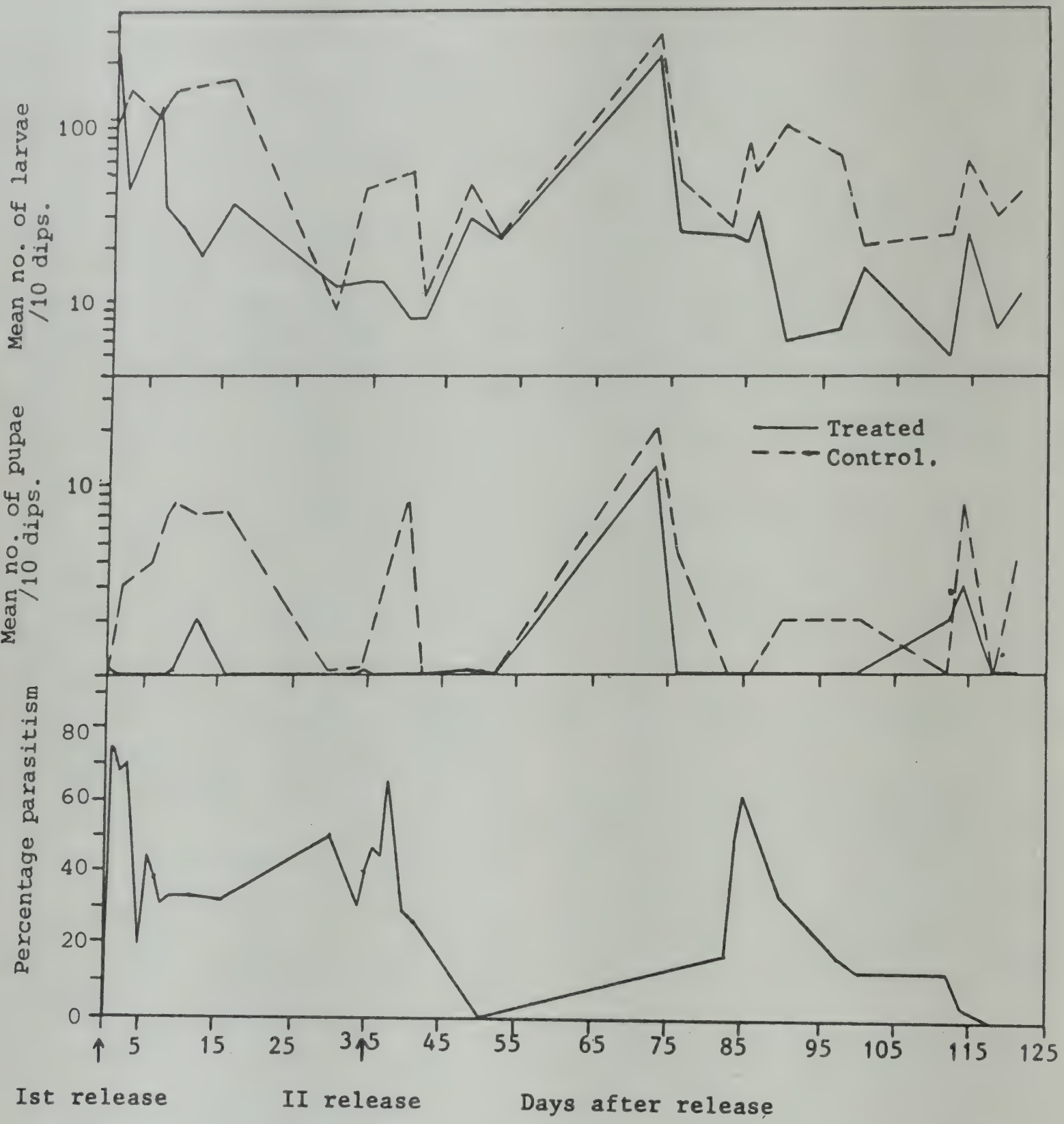


Figure 4.24. Effects of *R. iyengari* on Anophelines and culicines breeding in grass land.

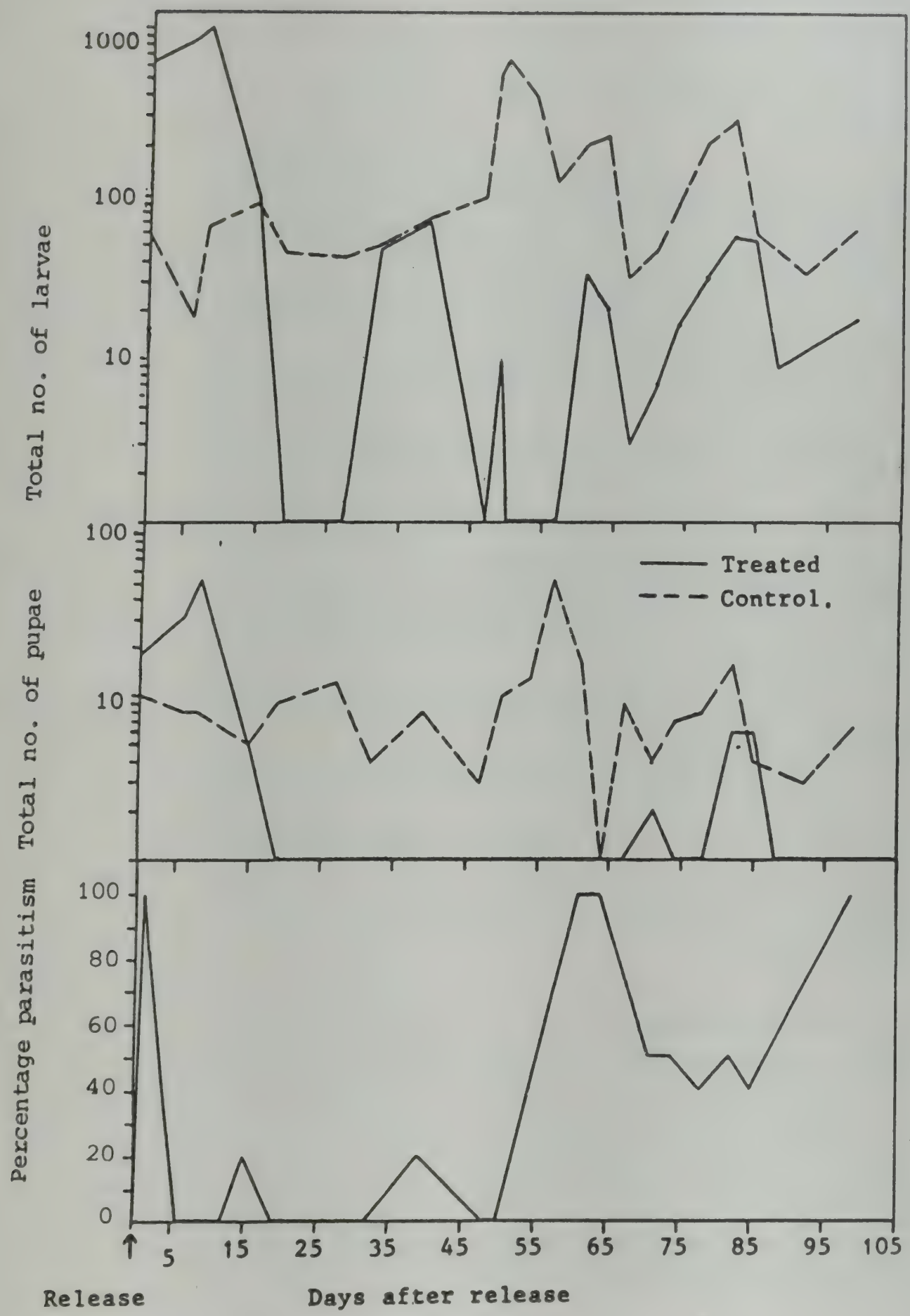


Figure 4.25. Effects of *R. iyengari* on *Aedes albopictus* breeding in treeholes.

results (Table: 4.4) indicate that the nematode treated habitats have high levels of salinity and conductivity and this was maximum in tree holes. The results of the field evaluation clearly show that *R. iyengari* has got the capability to survive in certain polluted sites.

4.15. Colonization of Mesocyclops leuckarti and its subsceptibility to insecticides

The colony of the copepod species which is being maintained at the VCRC has been identified as *M. leuckarti* based on, i. the presence of a deep rounded notch in the hyaline plate of the last segment of the I antenna and ii. the bare inner margin of the caudal ramus.

Sterile field water, groundnut cake and algae are the ingredients of the culturing medium used at present which supports a dense population of healthy individuals. With the above culture condition, a good number of adults have been produced for the past 10 months without any necessity for bringing adults from the field for crossing with the laboratory population.

The susceptibility of *M. leuckarti* adults to 12 insecticides namely bavistin, sevin, zolone, nuvan, baygon, ekalux, rogar, endosulphon, anthio, butachlor, alphamethrin and cypermethrin has been studied. The Lc50 and Lc90 values of these insecticides to *M. leuckarti* are given in the Fig. 4.26. Cypermethrin was found to be the most effective insecticide requiring 0.00047 ppm for causing 50% mortality.

4.16. Studies on the Microsporidians:

Amblyospora indicola, is found to parasitize the larvae of *Culex sitiens* in nature. To know whether this microsporidian has an intermediate host to complete its life cycle, Copepods were collected from the field, where *C. sitiens* breeds and examined for the presence of spores. So far 5 collections were made and about 500 Cyclops were examined and none was positive for the presence of spores.

4.17. Studies on the parasitoids of muscoid flies:

To find out the natural parasitism rate puparia collected at weekly intervals are isolated and fly eclosed or parasite emerged puparia are discarded and intact puparia are kept in individual plastic containers for observation for a period of 60 days. The daily emergence of parasitoids and flies from the puparia are being recorded. The flies and parasitoids emerged from the puparia are *Musca domestica* L., *Stomoxys calcitrans* L., *Fannia* sp. and 4 species of Pteromalid pupal parasitoids viz. *Pachycrepoideus vindemiae* Perkins. *Splangia endius* Walker. *Splangia nigroanea* Curtis. *Splangia cameroni* Perkins. and one chalcid wasp *Dirinius himalayanus* West wood. The percentage of natural parasitism ranges from 0 to 39.47. A comparison of different methods used to estimate the natural parasitism by taking into account total puparia, intact puparia and live puparia is being attempted. Further studies are in progress.

At ptesent studies are being carried out on the biology of the parasitoid *Spalangia cameroni*. The parasitoid mated soon after emergence and the duration of mating ranged from 8–15 sec. (av. 11.66). When mated females were provided with 24–48 h. old puparia, they all started ovipositing even on the day of emergence. The parasitoids completed their life cycle within 19–27 days (24 ± 1.89). The egg stage lasted 24–48 h, whereas the larval and pupal durations varied from 9–14 days (av. 12.3) and 7–11 days (av.9.2) respectively.

The biocontrol efficiency was studied by exposing freshly emerged mated female parasitoids to the puparia of house flies in the following ratio of parasitoids and hosts viz., 1:20, 2:20, 3:20, 4:20 and 5:20. Daily fresh hosts were provided to the parasitoids till they died. The puparia were observed for the possible emergence of either parasitiod or house fly. The mortality of house flies in different ratios observed were 36.0%, 49.5%, 48.5%, 65.5% and 92.5% respectively. The study infers that the increase in parasitoid density increases the rate of control of house flies.

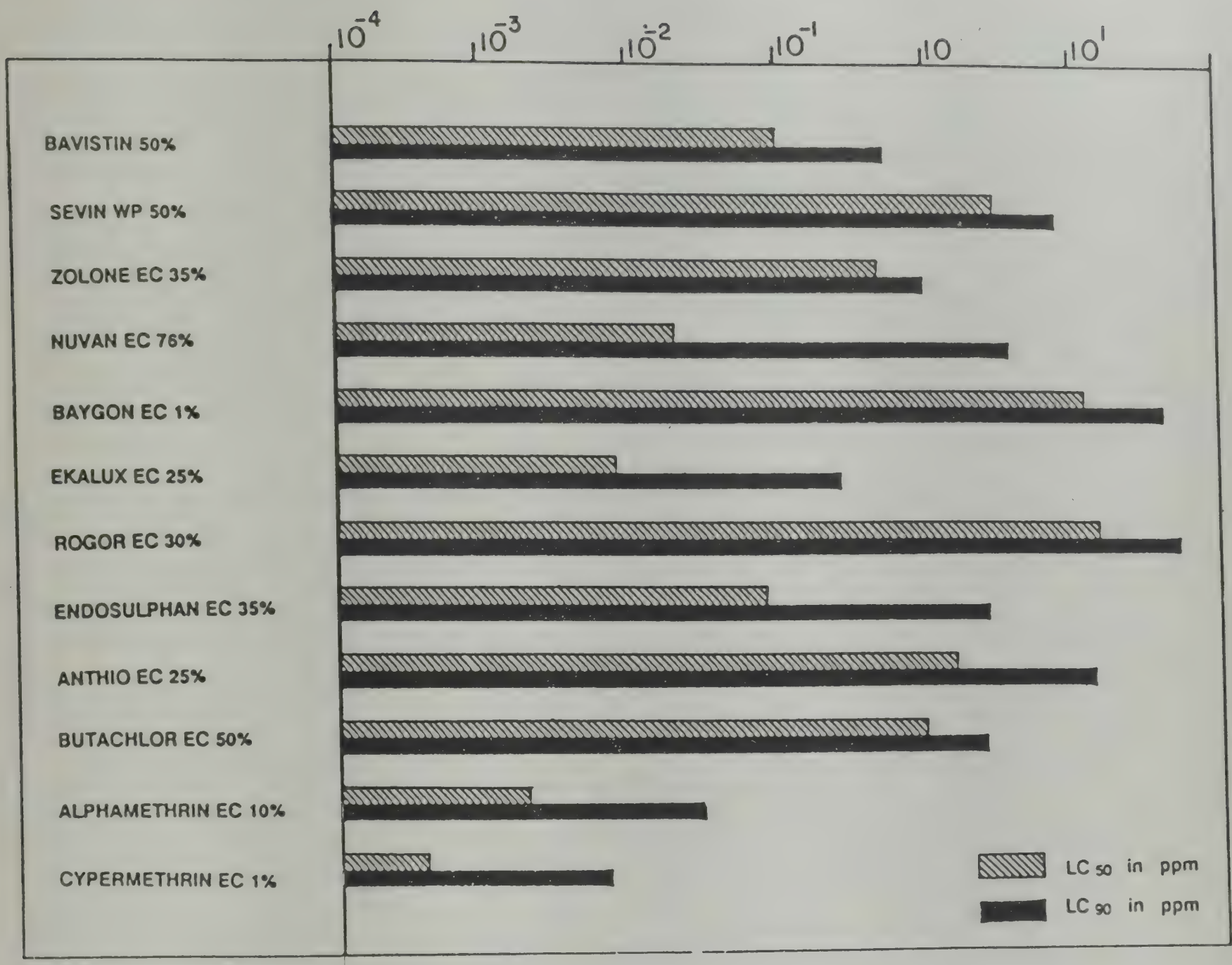


Figure 4.26. Relative toxicity of some insecticides against the adults of *Mesocyclops leuckarti*.

TABLE 4.4

Dosage of nematode application, level of pollution and nematode establishment in different mosquito breeding habitats

Breeding habitat	Extent covered	Dosage of application	pH of the water	Conductivity (micro mhos/cm.)	Dissolved oxygen (mg/l.)	Chloride (mg/l.)	% parasitism on different days after application	
							1st day	last day
Grass field	2000 M^2	5000 ppn/M^2	7.5	640	nil	100	1st day–74.0%	114th day–4.0%
Irrigation tank	200 M. length	800 post-parasites/M.	7.5	1200	3.2	152	25th day–12.5%	90th day–5.7%
Paddy field	4000 M^2	4000 ppn/M^2.	7.5	1000	14.4	158	2nd day–75.0%	22nd day–13.3%
Tree hole	7 Nos.	1:1, 1:2, 1:3, 1:4, 1:5 host-parasite ratio.	7.0–8.0 8.0	600–2800	2.8	110–1250	1st day–100.0%	99th day–100%

5. INSECTICIDES

Testing of different insecticides is an ongoing programme at the VCRC.

5.1. Adulticide:

5.1.1. OMS Compounds

The compounds OMS 3022 (Carbosulfan) and OMS 3040 (TIA-230) were evaluated for adulticidal activity against different mosquito vector species under laboratory conditions and the results are presented in Table 5.1. The compound OMS 3022 exhibited higher adulticidal activity than OMS 3040 and the LD_{50} value of this compound can be compared with that of conventional adulticides in use.

5.1.2. Cypermethrin, Deltamethrin and Propoxur

These three insecticides were also evaluated for their adulticidal efficacy and the results are presented in Table 5.2. Deltamethrin was found to be the most effective of these compounds.

5.1.3. Efficacy of insecticide spray deposits on different surfaces:

As the efficacy of the compound varies depending on type of surface, of late it was felt necessary to find out the effective life span of a compound on different surfaces before being exploited against vectors. OMS 3022 (25%EC), OMS 3040 (50%EC) and OMS 3002 (4% oil) formulations were tested on mud, thatch and cement surfaces and the results are presented in Table 5.3. OMS 3022 (25%EC) at the application rate of 0.4 g (ai)/m^2 caused more than 50% mortality for 19–21 weeks on mud surface and 12–16 weeks on thatch surface and only for 3 weeks on cement surface against all the three vector species. Whereas OMS 3040 (50%EC) at 2.0 g(ai)/m^2 was effective only on thatch surface giving more than 50% mortality for 5–8 weeks. OMS 3002 was also evaluated at a dosage of 1 gm/m^2 and it was found to be effective for only 1 week on Cement and mud surfaces and for 4 weeks on thatch surface for all the vector species.

Cypermethrin (1% EC formulation) when tested at 500 mg(ai)/m^2 was found to be effective 1, 10 and 7 weeks on mud, thatch and cement surfaces respectively. The Ridol formulation of cypermethrin (0.1% aqueous formulation) at the same rate of application was found to be effective for only one week and deltamethrin (0.02% EC) at the rates of applications, 10 and 25 mg(ai)/m^2 was found to be effective for only one week on all the three surfaces.

5.1.4. Evaluating the residual activity of insecticide deposits in insecticide impregnated nets:

Man-vector contact is one of the important factors in the disease transmission. Different methods are evolved to prevent or at least reduce the contact between man and vector. One such method is by using insecticide impregnated nets as curtains and bed nets. Mosquito nets (30 × 30 cm) impregnated with permethrin at 0.5 g(ai)/m^2, deltamethrin at 0.025 g(ai)/m^2 and cypermethrin at 0.5 g(ai)/m^2 were evaluated for residual activity against three vector species, viz., *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* at weekly intervals (Table 5.4).

Permethrin, deltamethrin and cypermethrin impregnated nets were found to cause more than 50% mortality for 44, 36 and 34 weeks respectively against all the three vector mosquitoes. It was demonstrated that the residual activity of these nets continued even after they were subjected to washing six months after impregnation.

5.2. Larvicide:

5.2.1. Insect Growth Regulators (IGRs):

OMS-3031 (XRD-473), a substituted urea compound was evaluated for emergence inhibition activity against *Culex quinquefasciatus*, *Aedes aegypti*, *Anopheles stephensi*, *Toxorhynchites splendens* and *Musca domestica* in the laboratory and against *Cx. quinquefasciatus*, *Ae. aegypti* and *M. domestica* in the field. This compound was effective in inhibiting the emergence of all the three vector mosquito species. When compared with fenoxycarb, methoprene and diflubenzuron, it was observed that OMS-3031 was comparatively more active. (Tables 5.5 and 5.6).

TABLE 5.1

Adulticidal activity of OMS 3022 and OMS 3040

Compound	Species	LD_{50} (ug/cm²)	Regression equation
OMS 3022	*Cx. quinquefasciatus*	6.652	Y = 1.51 + 1.83 log x
,,	*Ae. aegypti*	8.345	Y = 2.13 + 1.35 log x
,,	*An. stephensi*	5.192	Y = 2.68 + 1.40 log x
OMS 3040	*Cx. quinquefasciatus*	186.7119	Y = –6.003 + 2.104 log x
,,	*Ae. aegypti*	204.5946	Y = –1.126 + 1.151 log x
,,	*An. stephensi*	114.3381	Y = 1.62 + 0.714 log x

TABLE 5.2

Adulticidal efficacy of insecticides against mosquitoes

Compound	Test species	LD_{50} (ug/cm²)	Regression equation
Cypermethrin	*Cx. quinquefasciatus*	2.49	Y = 4.23 + 0.85 log x
,,	*Ae. aegypti*	1.65	Y = 4.65 + 0.69 log x
,,	*An. stephensi*	0.84	Y = 5.15 + 0.83 log x
Deltamethrin	*Cx. quinquefasciatus*	0.45	Y = 6.03 + 1.21 log x
,,	*Ae. aegypti*	0.52	Y = 6.02 + 1.54 log x
,,	*An. stephensi*	0.56	Y = 6.09 + 1.88 log x
Propoxur	*Cx. quinquefasciatus*	1.08	Y = 4.94 + 0.74 log x
,,	*Ae. aegypti*	1.23	Y = 4.85 + 0.72 log x
,,	*An. stephensi*	0.95	Y = 5.03 + 0.64 log x

TABLE 5.3

Residual activity of insecticides on different surfaces

Compound	Formulation	Rate of application g(ai)/m²	Species	Effectiveness (week)*		
				Cement	Thatch	Mud
OMS 3022	25%EC	0.4	*Cx. quinquefasciatus*	3	16	21
			Ae. aegypti	3	12	19
			An. stephensi	3	16	21
OMS 3040	50%EC	2.0	*Cx. quinquefasciatus*	1	8	1
			Ae. aegypti	1	8	1
			An. stephensi	1	5	1
OMS 3002	4%OIL	1.0	*Cx. quinquefasciatus*	1	4	1
			Ae. aegypti	1	4	1
			An. stephensi	1	4	1
Copermethrin	1% EC	0.5	*Cx. quinquefasciatus*	9	10	1
			Ae. aegypti	5	9	1
			An. stephensi	10	10	1
Cypermethrin	0.1% aqueous	0.5	*Cx. quinquefasciatus*	1	1	1
			Ae. aegypti	1	1	1
			An. stephensi	1	1	1
Deltamethrin	0.02% EC	0.01	*Cx. quinquefasciatus*	1	1	1
			Ae. aegypti	1	1	1
			An. stephensi	1	1	1
Deltamethrin	0.02% EC	0.025	*Cx. quinquefasciatus*	1	1	1
			Ae. aegypti	1	1	1
			An. stephensi	1	1	1

* › 50% mortality.

TABLE 5.4

Results of residual activity of insecticide impregnated nets against vector mosquitoes (in weeks)

Name of the insecticide	Dosage mg (ai)/m^2	*Cx. quinque-fasciatus*	*Aedes aegypti*	*Anopheles stephensi*
Permethrin	500	44	44	44
Deltamethrin	25	36	36	36
Cypermethrin	500	34	38	34

TABLE 5.5

Laboratory evaluation of OMS 3031 for IGR activity against mosquitoes and houseflies

Species	Regression equation	EI_{50} (mg/l)	EI_{90} (mg/l)
Cx. quinquefasciatus	$Y = 8.51 + 0.38 \log x$	9.00×10^{-5}	2.69×10^{-3}
Ae. aegypti	$Y = 8.50 + 0.38 \log x$	1.10×10^{-4}	3.06×10^{-3}
An. stephensi	$Y = 10.61 + 0.67 \log x$	2.23×10^{-4}	1.52×10^{-3}
T. splendens	$Y = 19.75 + 1.75 \log x$	2.14×10^{-4}	4.46×10^{-4}
M. domestica	$Y = 6.26 + 0.51 \log x$	8.29×10^{-2}	1.04

TABLE 5.6

Effect of IGR OMS 3031 on pupae of three vector mosquito species

Species	% EI at different dosages (mg/l)		
	0.01	0.1	1.0
Cx. quinquefasciatus	58	75	94
Ae. aegypti	34	70	82
An. stephensi	14	18	45

On comparison, OMS-3031 was found to be 15 and 18 times more effective than S-21149 against *Cx. quinquefasciatus* and *An. stephensi* respectively. EI_{50} of this compound and that of OMS-3009, OMS-3013, and OMS-2015 proved that all the IGRs are equally effective. As observed with other IGRs this compound was less effective on pupae (Table 5.6). As *T. splendens* was affected at lower doses in the laboratory, OMS 3031 has to be judiciously used in controlling *Ae. aegypti* in habitats where *T. splendens* is used as predator.

OMS-3031 was evaluated in cesspits, cement tanks, cesspools and drains against *Cx. quinquefasciatus* at three different doses (i.e., 0.01, 0.1 and 1.0 mg/1) and the results are graphically presented (Fig. 5.1). In cesspits this compound was found to be effective (80% EI) for 10 days at dosage 0.1 mg/1. When tested at lower dosage of 0.01 mg/1 only 5 days control was obtained. Complete emergence inhibition was observed for 5 days at the higher dose of 1.0 mg/1. In contrast, relatively lower dosage (0.1 mg/1) was applied in the cement tank but 100% EI was observed for 10 days. In drains, only 12 days of control was obtained at the higher dose of 1.0 mg/l. Like other IGRs, this compound was also more effective in clear water than polluted water.

More than two weeks control of *Ae. aegypti* was observed in cement tanks at the concentration of 0.1 mg/1, whereas at the lower dose of 0.01 mg/1 this compound was effective for less than a week (Fig. 5.1).

Heavy larval mortality, malformation of pupae resulting either in immediate death or delayed mortality due to incomplete emergence of adults from pupal cuticle was observed during the period of effectiveness.

Though the effectiveness of this compound is limited to freshwater, it can be incorporated in IVM programme. There are always some situations where water is clean but fish can not be used either due to low volume of water or due to unwillingness of people. In such situations IGRs can play useful role. Nonetheless, viewing the lethal effect of this compound on *T. splendens* there is imperative necessity for further study of this compound on non target organisms before being used in IVM programme.

OMS 3031 was evaluated for emergence inhibition activity against *M. domestica* in the laboratory and the results are presented in Table 5.5. The field evaluation of OMS 3031 against *M. domestica* is in progress.

5.2.2. OMS 3002, Cypermethrin, Deltamethrin and Propoxur:

The results of the larvicidal activity of OMS 3002 (4% oil formulation) against mosquito vector species under laboratory conditions are presented in Table 5.7.

The 1% EC formulation of cypermethrin was tested for larvicidal efficacy against fourth instar larvae of different mosquito vector species under laboratory conditions at different concentrations by preparing the suitable stock solutions in rectified spirit. The results of the larvicidal efficacy are presented in Table 5.8. From the results it was found that *Cx. quinquefasciatus* is most susceptible to cypermethrin followed by *Ae. aegypti* and *An. stephensi.*

The 0.02% EC formulation of deltamethrin and 1% EC formulation of propoxur were tested for the larvicidal activity against the different mosquito vector species under laboratory conditions and the results are given in Table 5.8. Deltamethrin was found to be more effective than cypermethrin and propoxur.

5.3. Formulations:

5.3.1. Evaluation of insecticide impregnated paint formulations for residual activity:

Development of new insecticides is cost-prohibitive and time consuming. The conventional insecticides can be used in vector control programmes by developing suitable formulations which can be applied in hospitals, air-port area and other common areas where frequent spraying can not be envisaged. Therefore, to increase the life of the existing insecticides and to reduce the toxic effects on non-target organisms insecticide impregnated paints were developed. By this way, effective control of pest species is achieved, so that pests are controlled and at the same time the problem of resistance development is either curtailed or slowed down. Formulations developed using DDT, HCH, fenitrothion, bendiocarb and permethrin were

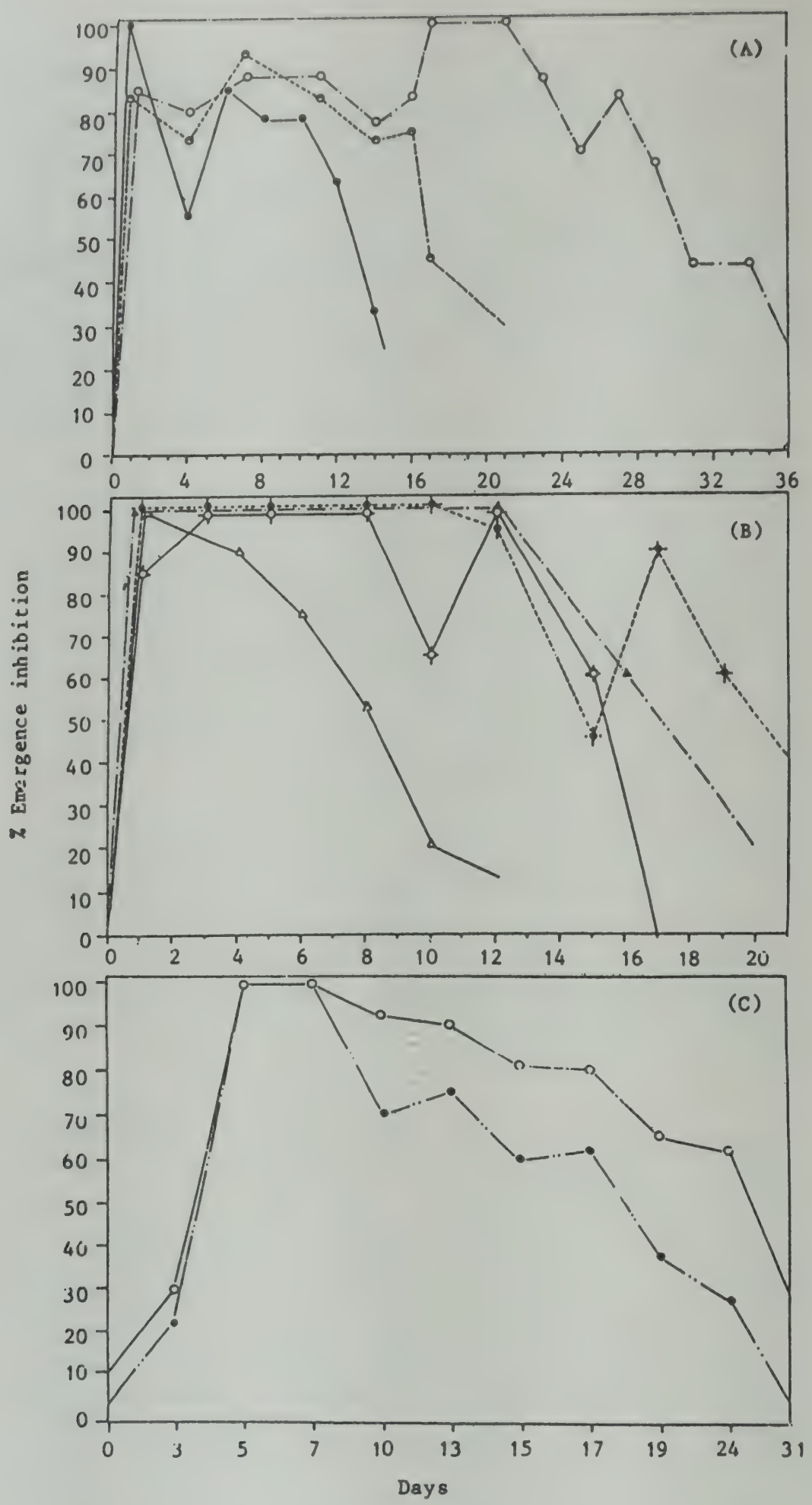


Figure 5.1. Field evaluation of IGR OMS–3031 against *C. quinquefasciatus* in (a) cess pit●–0.01 mg (ai)/1;◓–0.1 mg (ai)/1;○–1.0 mg (ai)/1; and (b) cement tank and drain-⊹–0.01 mg (ai)/1;✦–0.1 mg (ai)/1 (CT); △–0.1 mg (ai)/1 (drain); ▲–1.0 mg (ai)/1 (drain); and *Ae. aegypti* in (c) cement tank●–0.01 mg (ai)/1; ○–0.1 mg (ai)/1.

TABLE 5.7

Larvicidal activity of OMS 3002 (4% oil)

Species	Regression equation	EI_{50} (mg/l)	EI_{90} (mg/l)
Cx. quinquefasciatus	$Y = 12.39 + 1.43 \log x$	5.79×10^{-3}	1.41×10^{-2}
Ae. aegypti	$Y = 10.54 + 0.84 \log x$	1.47×10^{-3}	6.62×10^{-3}
An. stephensi	$Y = 6.03 + 1.10 \log x$	0.39	1.26

TABLE 5.8

Larvicidal efficacy of cypermethrin, deltamethrin & propoxur against different mosquito vectors

Compound	Test species	LC_{50} (mg/l)	Regression equation
Cypermethrin	*Cx. quinquefasciatus*	2.28×10^{-5}	$Y = 24.41 + 1.82 \log x$
"	*Ae. aegypti*	7.52×10^{-5}	$Y = 23.04 + 1.90 \log x$
"	*An. stephensi*	2.25×10^{-3}	$Y = 12.89 + 1.30 \log x$
Deltamethrin	*Cx. quinquefasciatus*	6.30×10^{-5}	$Y = 13.07 + 0.83 \log x$
"	*Ae. aegypti*	5.19×10^{-5}	$Y = 16.81 + 1.20 \log x$
"	*An. stephensi*	4.79×10^{-3}	$Y = 10.94 + 1.11 \log x$
Propoxur	*Cx. quinquefasciatus*	5.25×10^{-5}	$Y = 11.66 + 0.68 \log x$
"	*Ae. aegypti*	3.77×10^{-5}	$Y = 12.24 + 0.63 \log x$
"	*An. stephensi*	4.58×10^{-4}	$Y = 10.01 + 0.65 \log x$

TABLE 5.9

Evaluation of aerosol formulation of insecticides

Species	% Mortality in 24 hours	
	Pyrethrum (2.1%)	Propoxur (1%)
Cx. quinquefasciatus	100	100
Ae. aegypti	100	100
An. stephensi	100	100
M. domestica	97	97
P. americana	—	86

evaluated for residual activity on cement surface against three vector mosquito species, viz., *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* at weekly intervals and the results are presented in Fig. 5.2. The formulation INS/Paint/FB gave more than 50% mortality against all the three species for 49 weeks. The other 7 formulations except two were effective for 18–41 weeks.

5.3.2. Testing of Aerosol formulations against flying insects:

The method of using aerosols has been widely used for the control of large number of flying insects in a possible short time. The results of insecticide aerosol formulations tested in Peet-Grady chamber (1.8 × 1.8 × 1.8m) at 2ml/m^3 against different pest species are presented in Table 5.9. Aerosols of pyrethrum (2.1%) gave 100% mortality against all the three vector species and 97% mortality against *M. domestica*. Similarly 100%, 97% and 86% mortality against mosquito vectors, *M. domestica* and *Periplaneta americana* respectively were obtained with the aerosol formulation of propoxur (1%).

5.3.3. The effect of insecticides for knockdown activity against public health pests

The three insecticides, cypermethrin (0.1% aqueous formulation, known as Ridol), deltamethrin (0.02% EC) and propoxur (1.0% EC) were tested for knockdown activity against different public health pests at different stages in a Potter tower through which the formulations can be atomized to a fine space spray. The results of the insecticides showing the KD_{50} (ml/m^3) are presented in Table 5.10.

5.4. Synthesis of compounds for developing new control agents

Vector Control Research Centre has been actively engaged in synthesizing various substituted compounds for different types of biological activity against mosquito vector species for developing new non-persistent and selective chemicals with functional groups like amides, esters, ethers etc. The number of compounds synthesized during the year 1988 is given in the Table 5.11.

5.4.1. Development of compounds for Insect Growth Regulating activity:

Unlike conventional chemical larvicides which produce mortality due to their high neurotoxic effects, the IGRs induce various morphological and physiological abnormalities, thus causing pernicious effects leading to mortality in the treated insects. The development of insect growth regulators has been claiming much attention for selective control of insects of medical importance.

5.4.1.1. Diphenyl ethers:

Out of 35 substituted diphenyl ethers, three compounds, DPE-16, 19 and 28 have been found to be effective for insect growth regulating activity (VCRC Annual Report 1987). These compounds were tested against *Gambusia affinis*, a predatory fish at 1 mg/l concentration and found to be safe.

The three effective diphenyl ethers will be prepared in a larger quantity, formulated as EC and evaluated under Phase 2 of WHOPES against mosquito vector species in different breeding habitats and also against nontarget organisms like *Toxorhynchites splendens*, a predatory mosquito and *Gambusia affinis*, a predatory fish.

5.4.1.2. Diphenyl ureas:

Out of 29 compounds synthesized and evaluated, 11 compounds have been found to be effective for IGR activity. The results of DPU-3, 4,6,7,8 and 12 have been presented in earlier reports (VCRC Annual Reports 1986 and 1987). The results of DPU-24, 26, 27, 28 and 29 are presented in Table 5.12. Of the five diphenyl urea compounds tested for IGR activity, DPU-26 was found to be effective against *Cx. quinquefasciatus* and *An. stephensi* at concentrations lower than 0.5 mg/l. These compounds will be suitably formulated and tested in small scale field trial in various breeding habitats.

5.4.1.3. Amides:

During the year 1988, 29 substituted amides having structure analogous to A-23, (Piperidinyl 2,4-dichlorophenoxy acetamide) an amide reported to have IGR activity at relatively higher concentration of 5 mg/l have been synthesized and the preliminary screening of these compounds for IGR activity at 1 mg/l against different vector species showed that six compounds (A-91, 92, 93,

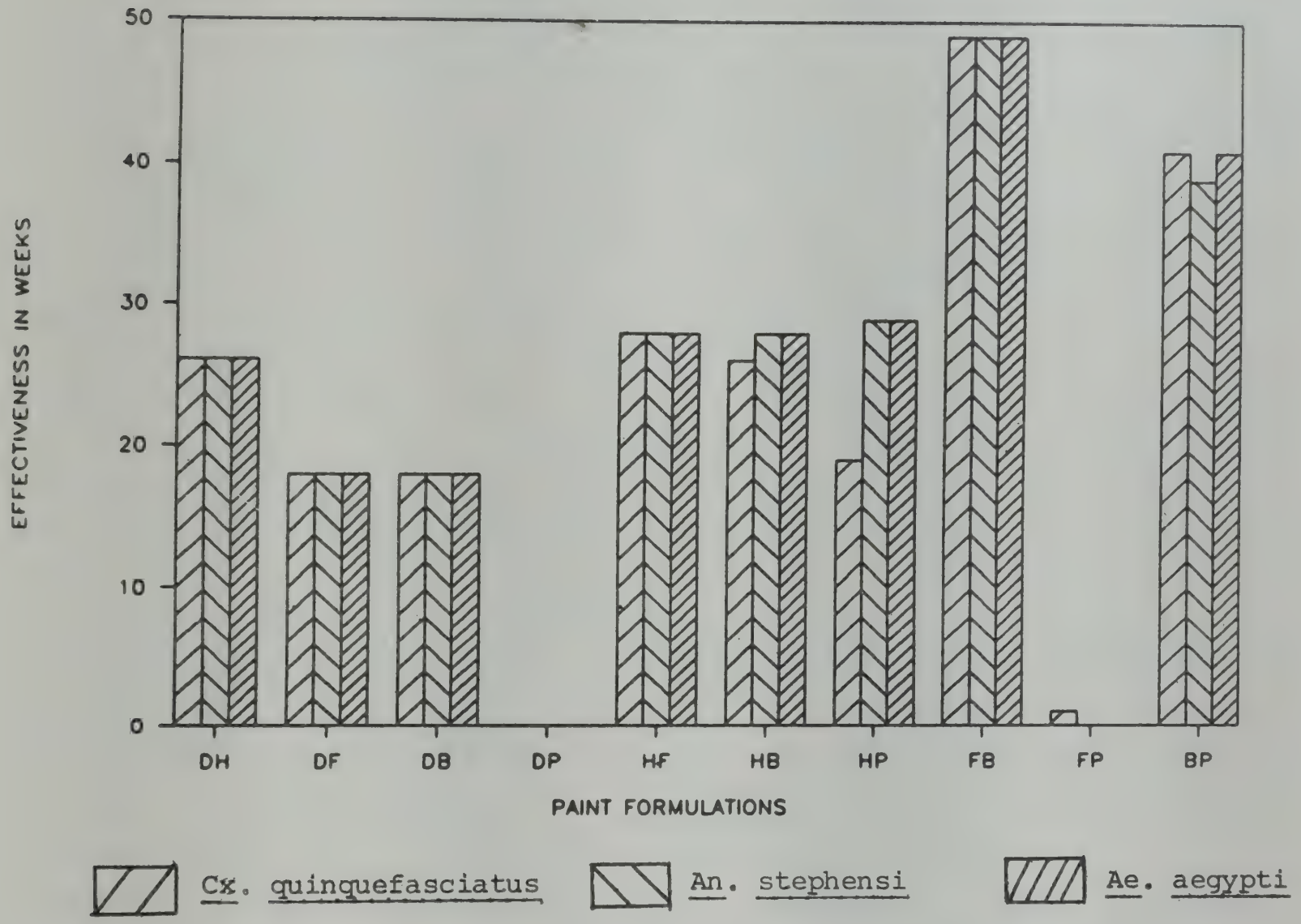


Figure 5.2. Residual efficacy of paint formulations.

TABLE 5.10

The effect of space spray with certain insecticides against public health pests for knock down activity

Name of the Insecticide	Species / Stage	KD_{50} in (ml/m^3)	Regression equation
Cypermethrin (0.1% AQ.) (Ridol)	MOSQUITOES (Adult females)		
	Cx. quinquefasciatus	0.09	Y = 5.08 + 0.55 log x
	An. stephensi	0.08	Y = 5.15 + 0.57 log x
	Ae. aegypti	0.17	Y = 4.65 + 0.66 log x
	COCKROACHES		
	P. americana (female)	7.44	Y = – 4.62 + 2.23 log x
	P. americana (nymph)	9.34	Y = – 13.82 + 4.15 log x
	P. americana (male)	7.66	Y = – 6.87 + 2.73 log x
	HOUSEFLY		
	M. domestica (lab strain)	1.23	Y = – 0.0096 + 2.02 log x
	M. domestica (field strain)	1.09	Y = 1.33 + 1.53 log x
Deltamethrin (0.02% EC)	MOSQUITOES (Adult females)		
	Cx. quinquefasciatus	0.03	Y = 5.56 + 0.53 log x
	An. stephensi	0.03	Y = 5.89 + 0.73 log x
	Ae. aegypti	0.02	Y = 5.46 + 0.71 log x
	COCKROACHES		
	P. americana (female)	8.66	Y = – 12.47 + 3.92 log x
	P. americana (nymph)	8.06	Y = – 12.26 + 3.93 log x
	P. americana (male)	7.83	Y = – 4.42 + 2.16 log x
	HOUSEFLY		
	M. domestica (lab strain)	2.77	Y = 1.049 + 1.19 log x
	M. domestica (field strain)	3.98	Y = – 0.29 + 1.43 log x
Propoxur (1% EC)	MOSQUITOES (Adult females)		
	Cx. quinquefasciatus	0.45	Y = 2.86 + 1.43 log x
	An. stephensi	0.03	Y = 5.57 + 0.49 log x
	Ae. aegypti	0.16	Y = 4.71 + 0.65 log x
	COCKROACHES		
	P. americana (female)	5.68	Y = – 3.22 + 2.04 log x
	P. americana (nymph)	7.67	Y = – 9.85 + 3.42 log x
	P. americana (male)	5.95	Y = – 6.09 + 2.71 log x
	HOUSEFLY		
	M. domestica (lab strain)	6.35	Y = 7.51 + 3.01 log x
	M. domestica (field strain)	2.45	Y = 1.14 + 1.20 log x

TABLE 5.11

List of compounds synthesized during 1988

Chemical class	Number of compounds synthesized	Type of activity
Amides (structure analogous to A-23)	29	IGR
Amides of N-Benzoyl glycines	37	IGR
Acetates	10	Oviposition attractancy/ repellency
Pyrethroid esters	15	Larvicidal & Adulticidal

TABLE 5.12

The IGR activity of the effective Diphenyl ureas

Code No.	EI_{50} (mg/l) against *Cx. quinquefasciatus*	*Ae. aegypti*	*An. stephensi*
DPU–24	0.4872	0.7404	1.3038
DPU–26	0.4433	0.5649	0.2417
DPU–27	0.6290	0.6870	0.4707
DPU–28	0.5313	0.7399	0.4247
DPU–29	0.6444	0.7804	0.8850

TABLE 5.13

Oviposition attractancy/repellency of acetates

Acetate Code No.	Oviposition Active Index	Test Concn. (mg/l)
AC– 7	+ 0.31	1.0
AC–21	+ 0.36	0.1
AC– 2	+ 0.81	0.1
AC– 4	– 0.30	1.0
AC– 9	– 0.64	1.0
AC–10	– 0.41	1.0
AC–16	– 0.44	0.01
AC–19	– 0.35	1.0

94, 98 and 104) were found to be effective. These compounds are being tested at lower concentrations.

5.4.1.4. Amides of N-Benzoyl glycine:

Substituted amides of N-benzoyl glycine (hippuric acids) have been prepared by reacting the aromatic acid chloride with glycine in good yield. The different substituted N-benzoyl glycines were reacted with substituted anilines to yield the amides in satisfactory yield (>60% yield). These compounds have been recrystallized in hot chloroform. The structures of the compounds were in agreement with IR and NMR spectral data. These compounds are being screened for IGR activity.

5.4.2. Development of compounds for oviposition attractancy/repellency:

Mosquito control in urban area poses special problem due to the enormity of breeding habitats. The breeding habitats are widely distributed that detection and treatment of each habitat involves not only larger manpower but also cost-prohibitive. The oviposition attractant can play an useful role by directing the ovipositing females to few desired sites which can conveniently be taken care of by appropriate control methods. The attractant can also play an important role in monitoring mosquito population. VCRC continues to synthesize and evaluate the compounds for oviposition attractancy/repellency.

5.4.2.1. Acetates:

Acetates of various types of alcohols (saturrated straight chain and branched alcohols, unsaturated straight chain and unsaturated branched alcohols) have been prepared and tested for oviposition attractancy/repellency against gravid females of *Culex quinquefasciatus*. So far 33 acetates have been prepared and 23 compounds have been tested. Out of 23 compounds, two compounds AC-7 and AC-21 were found to be effective for oviposition attractancy and six compounds AC-2, 4, 9, 10, 16 and 19 were effective for oviposition repellency. The results are presented in Table 5.13.

5.4.3. Development of compounds for adulticidal and larvicidal activity.

5.4.3.1. Pyrethroid esters:

So far 32 substituted pyrethroid esters have been synthesized by reacting various aromatic acid chlorides with substituted benzyl alcohols and tested for larvicidal and adulticidal activities. Only SPE-7 was found to have significant larvicidal activity at 1 mg/l.

Recently, another class of pyrethroid esters was developed by reacting 2-phenoxy-3-methyl butanoic acids with various substituents in the phenyl ring with substituted benzyl alcohols. So far five pyrethroid esters of this type have been synthesized, purified by column chromatography. The structures of these compounds have been confirmed by both IR and NMR spectral data. These compounds will be tested for larvicidal and adulticidal activities.

5.5. Development of Controlled release formulations of larvicides.

Controlled release formulations of insecticides have been given the prime importance in recent years to extend the effective duration of chemical larvicides thereby minimizing the cost of the control operations.

5.5.1. CMC Controlled release formulations:

Carboxymethyl cellulose, a cheap, highly biodegradable and chemically modified natural polymer is chosen for the development of controlled release formulation. The difficulties frequently encountered in this type of formulation, viz., immediate gelling with gellant material and extensive swelling of finished formulation have been overcome by modifying the conditions of cross-linking. The formulated material is made to float by suitably incorporating low density materials. CMC formulations with larvicides fenthion and temephos (organophosphorus larvicides) have been developed. The initial studies under laboratory conditions related to the release profile using HPLC technique have revealed that this formulation is effective for more than 45 days with the concentration of the active ingredient ranging between 0.2 and 1 mg/l. A small scalè field trial will be carried out using this formulation.

5.6. Development of suitable techniques for analysing antimalarial drugs using HPLC:

The analysis of the antimalarial drugs like

chloroquine and primaquine plays an important role in the quality control of drugs distributed through various primary health centres in malaria endemic areas as well as in detecting the drugs in very low quantities in biological samples, viz., blood and urine.

At Vector Control Research Centre, an analytical method for estimating Chloroquine and Primaquine using the Shimadzu HPLC was standardized. The Sigma grade chloroquine phosphate and primaquine phosphate were used for the study. The Zorbax ODS reverse phase column was used. The phosphate buffer in acetonitrile was used as the mobile phase with perchlorate as the counter ion. The UV absorption at 254 and 340 nm and fluorescent detection (for chloroquine: Exc: 335 and Emi: 370 nm and for primaquine Exc: 350 and Emi: 450 nm) were used. In the case of chloroquine analysis, fluorescence detector was more sensitive than UV detector and in the case of primaquine, analysis using UV absorption at 254 nm was found to be optimum condition.

Four samples of chloroquine and primaquine tablets were analysed for their quality and the results are presented in Tables 5.14 and 5.15.

On the importance of Historical studies.

"The Debris of broken systems and exploded dogmas form a great mound, a Monte Testaccio of the shards and remnants of old vessels which once held human beliefs. If you take the trouble to climb to the top of it, you will widen your horizon, and in these days of specialized knowledge your horizon is not likely to be any too wide"

OLIVER WENDELL HOLMES.
—Paul Russel in Man's Mastery of Malaria.

TABLE 5.14

Analysis of Chloroquine base by HPLC using RF–535 Fluorescence Detector

Sample No.	Source	Batch No.	Chloroquine By HPLC (mg)	Base Expected (mg)
C1	SRIQUIN	SRT 138	132.01	150
C2	NMEP (Orissa State) (Rabanaguda PHC)	6037	133.22	150
C3	UNICEF	50050578	135.94	150
C4	Tamil Nadu Drugs	034	133.68	150

TABLE 5.15

Analysis of Primaquine base by HPLC using UV Detector at 254 nm

Sample No.	Source	Batch No.	Primaquine By HPLC (mg)	Base Expected (mg)
P1	NMEP (Orissa State) (Rabanaguda PHC)	050128	2.243	2.5
P2	NMEP (Orissa State) (DMO, Jeypore)	B 901433	6.387	7.5
P3	”	169019	2.019	2.5
P4	UNICEF	—	2.043	2.5

6. OTHER STUDIES

6.1. Rearing and Colonization:

6.1.1. Cyclic colonies of the following insects are being maintained in the centre.

Mosquitoes : *Culex quinquefasciatus*, *Culex tritaeniorhynchus*, *Culex sitiens*, *Culex (Lutzia) fuscanus*, *Anopheles stephensi*, *Anopheles culicifacies*, *Anopheles subpictus*, *Armigeres subalbatus*, *Aedes aegypti*, *Aedes albopictus*, *Toxorhynchites splendens* and *Mansonia annulifera.*

Housefly : *Musca domestica*

Cockroach : *Periplaneta americana*

Parasitic wasps : *Pachycrepoideus vindemmiae*, *Dirhinus himalayanus*, *Spalangia sp.* and *Tetrastichus hagenowii.*

6.1.2. Colonization of sand fly, P. papatasi:

Efforts are being continued to establish a cyclic colony of *P. papatasi.* The colony at present is in F^{10} generation. Though the sand fly is adapted to feed and oviposit under laboratory condition, due to death of females soon after oviposition and infestation of fungi in the rearing medium, the number of progeny obtained in each generation did not exceed 100. To overcome such constraints gravid females are being maintained in a humidity chamber which showed improvement in the survival rate. To avoid fungal growth larvae are being reared in sterilized field mud. When sterilized cowdung was used the larvae showed better growth rate than in field mud. Further observations are in progress.

6.1.2. Studies on the biology of sand fly:

Studies on the biology of sand fly, *P. papatasi* are being continued. The immature duration of different stages observed were as follows: I instar 3–5 days, II instar 5–8 days, III instar 4–6 days and IV instar 6–18 days. The pupal stage lasted for 7–12 days. The survival of larvae ranged from 57.5%–72.9% (Av. 63.7%). The pupation rate had varied from 56.5–87.1% and emergence rate from 53.4–87.0%. The sex ratio of male to female was 1:1.2. In all sets of experiments males had emerged first and were followed by females.

The mating behaviour also was studied and about 87% of the females were successfully inseminated when they were 3 days old. When the effect of light and space on mating was studied mating occured both in the presence and absence of light. The space also did not influence the insemination due to its stenogamic behaviour.

The role of sand fly in the transmission of leishmaniasis is well known. The spread of this disease to nonendemic area needs detail on the sand fly and its distribution. Hence a study has been initiated on the ecology and behaviour of sand fly in Pondicherry.

To determine the species composition and seasonal abundance, sand flies are being collected at weekly intervals from habitats such as human dwellings, cattle shed, bushes, tree holes and termite holes. So far a total of 3,274 sand flies comprising of 2 genera were collected. The species obtained are as follows in their order of abundance: *Phlebotomus papatasi* (37.41%), *Sergentomyia punjabensis* (21.59%), *S. clydei* (15.76%), *S. bailyi* (12.89%), *P. argentipes* (11.84%), *S. indica* (0.32%) and *P. colabaensis* (0.19%).

To screen the breeding habitats of sand flies, soil samples were collected from habitats like rodent burrows, tree holes, cattle shed and human dwellings and were examined for the presence of immatures. Of the 92 soil samples tested none was positive for sandfly breeding. However, when traps were set *P. papatasi* and *S. babu* were obtained from rat holes and termite holes respectively.

Regarding resting behaviour, it was noticed that *P. papatasi* was found to occur only in indoor and *P. argentipes* was seen both in indoor and outdoor collections. The density of *P. papatasi* was highest during summer especially in May (13.5/MHR) whereas *P. argentipes* showed a peak (29.22/MHR) during wet season (September).

To investigate the peak biting activity both

man biting and cattle biting collections were made from 18.00 h.–06.00h. at monthly intervals. It was seen that *P. papatasi* was relatively more endophagic whereas *P. argentipes* was exophagic.

The number of *P. papatasi* biting (44/man/night) was found to be higher during summer and relatively low during winter (5/man/night) corresponding figures for *P. argentipes* were 8 and 16 respectively.

The host feeding pattern is also being studied. To find out the source of blood meal each freshly fed female collected from resting sites is crushed in Whatman filter paper and is being subjected for precipitation test. About 95% of *P. papatasi* females were positive for human blood when tested.

Further studies are in progress.

6.2 Vector Gentics.

6.2.1. Studies in Anopheles subpictus complex

It has been reported (Ref. Annual Report 1987–88, p. 69) that *A. subpictus* consists of 3 sibling species, 2 fresh water breeding and one salt water breeding.

Studies on the distribution pattern of *A. subpictus* in inland and coastal localities of Pondicherry has shown yet another new species in *A. subpictus* complex.

All the four species of the complex could be differentiated by their egg morphology, larval mesothoracic hairs, pupal chaetobaxy and marking on the adult wings.

Cytological evidences from male mitotic karyotype substantiates the morphological findings. In *A. subpictus* the sex chromosomes are acrocentric. The long arm of the Y chromosome varies in length in the four species.

Salinity tolerance tests have shown that species B and D have a higher tolerance than species A and C.

It has also been found that in riverine pools *A. subpictus* breeds along with *A. culicifacies*, and in paddy fields with *A. vagus*. All the three species could be identified at the pupal stage and this can be confirmed after emergence. Male mitotic chromosome of the three species also differ.

6.2.2. Biology and genetics of field susceptible Culex quinquefasciatus to W. bancrofti.

As genetic evidence show that field susceptible *C. quinquefasciatus* have a definite genetic pattern, using eye color mutants, the following studies were undertaken to study the fecundity, fertility, the stages of filarial larvae in the infected mosquitoes, the number of each stages, age of the mosquito and if possible to select a refractory line using the index of susceptibility formula. If the index was more than 0.71 it was susceptible, and if less than 0.10 non susceptible.

Seven localities were selected for this study from Pondicherry. Infection and infectivity rate varied between 2.6–14.70% and 0.03–1.80% respectively.

It was observed in these collections that 8 percent of the infected mosquitoes had L_1 and L_2 stages. These mosquitoes laid eggs from the 3rd day –10th day after collection. Those which showed delay in egg laying had a larger proportion of arrested larvae and very few developing larvae. The progeny of these mosquitoes also showed a different recombination rate with eye color mutants. Tentatively they are considered as non-susceptible lines. Experimental infection studies will confirm the status of these lines.

6.2.3. Fenthion resistance in Culex quinquefasciatus

A pure homozygous resistant (RR) and a susceptible (SS) line for fenthion have been established in *C. quinquefasciatus* using the discriminating dosages. In every generation RR and SS are checked for their purity.

In the resistant line, two lines were maintained; one being treated with fenthion in every generation (treated) and the other without treatment (untreated). After five generations, the egg fecundity was increased in the untreated line whereas it decreased in the treated line. Egg fertility was also showing partial hatches in the treated line. It suggests that continuous exposure to fenthion will affect the egg fertility and fecundity.

The field population of *C. quinquefasciatus* from 7 different localities in Pondicherry were characterized according to the three genotypes, Resistant homozygotes, Susceptible homozygotes and heterozygotes.

Since fenthion resistance is semi-dominant, the overall data shows that 71% of *C. quinquefasciatus* are resistant to fenthion. But data from individual localities vary in their resistant status. Existence of heterozygotes and pure susceptibles suggest that fenthion could be used as a larvicide in Pondicherry.

6.3. Effect of insect growth regulators on vector biology.

The effect of IGR–OMS 3031 on the hatchability of eggs of three species of vector mosquitoes (*Cx. quinquefasciatus*, *An. stephensi* & *Ae. aegypti*) was studied at varying concentrations and it was found to result in either reduction of normal hatching or induction of abnormalities during the process. Freshly laid eggs were found to be more susceptible than older ones (12–18 hrs). Ovipositional behaviour was also severely hampered when the water surface was treated with this compound at higher doses, finally resulting in the death of the gravid females. Exposure of fourth instar larvae for short duration of time to medium containing 0.1 ppm of the compound led to 100% emergence inhibition. The impact of the growth regulator on the fertility of emerged individuals was also studied and males were found to be more sterile than the females.

The studies on the lethal and sub-lethal effects of this IGR on the growth and development of larvae and the associated physiological events such as Chitin synthesis inhibition, histological changes during the process and other histopathological studies are in progress.

6.4. Expanded polystyrene beads for controlling mosquito breeding in unused polluted wells in Bangalore City.

In Bangalore city mosquito nuisance is mainly due to the common house mosquito *Culex quinquefasciatus* and it was found to be the major dominant species in all seasons of the year. During a survey conducted in Bangalore city it was found that disused wells are one of the potent sources of mosquito breeding especially for the urban mosquito, *C. quinquefasciatus*. Provision of water supply through pipes has rendered hundreds of domestic wells unnecessary in Bangalore city. In some places due to sewage leakage and accidental linkage with underground sewerage system, the well water is mixed up with sewage water and forms ideal breeding ground for *C. quinquefasciatus*. Regular spraying of insecticide is not advisable as they may pollute the ground water. Expanded polystyrene beads (EPS) were applied in these wells as an alternate method and its effectiveness was monitored in selected wells to study the efficacy of EPS.

A total of 27 wells were selected for treating with EPS and 5 wells were kept as control wells. The water surface was covered over by one inch thick layer of EPS beads. After application, the treated and control wells were monitored at fortnightly intervals for any breeding. For checking breeding in EPS treated wells, a layer of bead was opened up and 2 bucket samples taken. Then the beads again covered up the gap later. Emergence traps were not fixed to study the emergence of mosquitoes from the treated wells as the traps will prevent the entry of ovipositing females.

In all the 27 wells observation was continued for two years and 4 months (June 1986 to September 1988). The EPS surface was found intact in all treated wells except some algal growth. Even in rainy months of June to November, the EPS surface was not disturbed. All treated wells were found negative which clearly indicates the effectiveness of EPS in preventing oviposition of mosquitoes since the treated wells were heavily breeding before treatment and the mean pretreatment densities of larval instars and pupae in the treated wells were 2130.3 and 282.2 respectively. But in control wells, the density of *C. quinquefasciatus* larval stages varied from 36 (September 1986) to 869 (April 1987) per bucket and the pupal density ranged from 14 (September 1986) to 200 (May 1987) per bucket. Mosquito breeding was observed throughout the year in all control wells.

Well survey was carried out from six areas of Bangalore city to assess the magnitude of mosquito breeding in wells. Out of 732 wells surveyed, heavy breeding of *C. quinquefasciatus* was found in 34

polluted wells (4.60%). Breeding in used wells is being controlled by releasing fish like *Gambusia* but in polluted unused wells EPS was found to be the best alternative for long term control. People prefer to retain the wells for any possible use later in case of water scarcity, a feature not uncommon in big cities. Here, EPS application provides an effective means to make these wells unsuitable for mosquito breeding. Since EPS is a non pollutant, the well water could be used for domestic use after removing EPS and making some arrangement for avoiding seepage of sewage into the wells. Due to the long term effect of EPS, insecticides as well as man-power could be saved by avoiding weekly spray of insecticide.

6.5 Studies on Armigeres subalbatus

The seasonal fluctuation in the density of *Armigeres subalbatus* was monitored through one year by routine fortnightly collections in indoor (human dwellings), outdoor and cattle shed resting sites at 7 fixed stations namely, Muthialpet, Mudaliarpet, Kosapalayam, Kottakuppam, Reddiarpalayam, Nainarmandapam and Thengathittu. The first three localities could be categorised as urban as they are contiguous with the town of Pondicherry, the next two as semi-urban and the last two as rural.

The relative resting density of *Ar. subalbatus* for the period January to December is summarised for all the seven stations and given in Table 6.5.1. Peak density occurred in February in all the three resting sites. Densities decreased during May and June after which build up in the population was noticed. Lowest density was observed in June. Dissections for the parity status of females also showed that the proportion of females that have completed two ovipositions was less during June and July and females having gone through four ovipositions occurred only during January and February.

A comparison of the resting densities summarised for the year is shown in Table 6.5.2 for the seven localities where the study was conducted. In all localities the resting density was high in outdoor, followed by cattle shed and indoor. The occurrence of *Ar. subalbatus* did not vary much between urban and semi-urban conditions. Septic tanks which are the major breeding source for this species are found not only in an urban area but also in a semi-urban condition. Thus the densities as observed in urban Kosapalayam (17.36 females/m.hour) did not vary much from that seen in semi urban Kottakuppam (19.44 females/m. hour), whereas in rural localities like Thengathittu and Nainarmandapam where much of the acreage is under cultivation and a near rural atmosphere prevails, the breeding sources are highly limited and consequently the density obtained is very low (1.00 female/m. hour). Thus the major constraint for the occurrence of this species is the availability of breeding source. Studies on the immatures are in progress to substantiate the observation made with adult populations.

Changes in the sex ratio of resting mosquitoes in different months are shown in Table 6.5.1. Males never outnumbered females at any time thoughout the year. The ratio of females over males, however, was high with the increase in density. It has to be mentioned that during certain months only females were caught in indoor and cattle shed resting sites.

Density of resting *Ar. subalbatus* was lowest in human dwellings. Cattle shed showed a slightly higher density whereas highest was in outdoor collections, (Table 6.5.1). Resting preference did not change with season, since throughout the year the proportion resting outdoors remained the highest followed by that in cattle shed and indoor. Males were no exception to this, as the proportion of males caught in the three resting sites also followed the same pattern. Thus the exophilic nature of this species is constant throughout the year.

Biting collections were done every fortnight between 18.00 hrs and 22.00 hrs in six stations. Indoor and outdoor man biting collections were done in Muthialpet, Mudaliarpet, Kottakuppam and Reddiarpalayam whereas indoor man biting and outdoor cattle biting collections were done in Thengathittu and Nainarmandapam. Biting densities obtained in various months are shown in Table 6.5.3.

Peak biting density occurred during January and February, when resting mosquitoes collected were also high. A peak in the biting density was seen again in July, the period during which resting

TABLE 6.5.1

Resting Density* and Sex Ratio** of *Armigeres subalbatus*

Month	Indoor		Outdoor		Cattle shed	
	Density	Sex ratio	Density	Sex ratio	Density	Sex ratio
Jan '88	0.5	2.1	35.4	5.3	3.8	11.4
Feb	1.0	3.3	45.3	3.6	11.5	20.1
Mar	0.6	2.4	32.8	2.1	5.4	10.7
Apr	0.4	4.3	18.9	2.0	0.7	only ♀
May	0.1	only ♀	11.1	2.3	0.4	6.0
Jun	0.1	only ♀	9.3	1.9	0	—
Jul	0.1	5.0	17.6	1.6	0.2	only ♀
Aug	0.1	only ♀	24.1	3.3	0.4	only ♀
Sep	0.3	only ♀	28.9	2.6	1.4	1.9
Oct	0.3	3.0	29.1	3.6	3.7	3.3
Nov	0.6	10.0	18.6	4.2	1.6	1.6
Dec	0.4	2.2	20.1	1.7	2.7	5.4

* females per man hour
** No. of females/No. of males

TABLE 6.5.2

Resting density of *Armigeres subalbatus* (females/m. hour) in different localities

	Indoor	Outdoor	Cattle shed	Average
Muthialpet	0.46	22.58	0.88	6.66
Mudaliarpet	0.10	8.58	1.17	2.71
Kosapalayam	0.92	56.38	5.25	17.36
Kottakuppam	0.71	58.03	9.48	19.44
Reddiarpalayam	0.13	19.30	0.67	5.46
Thengathittu	0.03	3.67	0.13	1.04
Nainarmandapam	0.30	2.36	0.79	1.00

TABLE 6.5.3

Biting density of *Armigeres subalabatus* (females biting a man or cattle/hour)

Month	Indoor (man)	Outdoor (man)	Outdoor (cattle)
Jan '88	3.4	5.6	0.3
Feb	1.7	5.5	0.3
Mar	1.9	4.8	0
Apr	0.7	2.7	0
May	0.3	0.5	0
Jun	0.1	0.4	0
Jul	1.2	5.8	0.1
Aug	0.2	1.1	0
Sep	0.3	2.4	0.1
Oct	0.1	1.2	0.1
Nov	0.2	0.8	0
Dec	0.2	1.0	0

Theoretically, the control of malaria is relatively simple, but as a practical undertaking it has been found extremely difficult.

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population also started building up after a trough in summer. The species is predominantly exophagic though a certain production enter human dwellings for feeding. It also prefers to bite man rather than cattle. However, further studies are in progress to confirm this observation.

Biting rhythm of the species was studied by conducting whole night man biting collection in one station between 18.00 h and 6.00 h. Peak biting activity was observed at 18.00–19.00 hrs. when the proportion of females biting man was as high as 0.7. In the same locality, collection was done between 17.00–18.00 hrs. during the period January to March through which it was seen that the proportion biting at this hour was low (0.2) as compared to that between 18.00–19.00 hrs. when it was 0.6.

6.6 Studies on Culex tritaeniorhynchus

Routine observations are being carried out on *Culex tritaeniorhynchus*, because of its importance as a vector of Japanese encephalitis in Pondicherry.

Maximum resting density of 7.68, 21.36 and 38.61 was observed indoors, outdoors and in cattle sheds respectively in the month of October. The next highest density was recorded in the month of July. The resting density ranged from 1.00 in May to 7.68 in October indoors, from 2.04 in June to 21.63 in October outdoors and from 2.06 in May to 38.61 in October in cattle sheds. The average resting density was higher in villages.

Peak indoor and outdoor man biting density was recorded in October and cattle biting density in August followed by October. The man biting density ranged from 0.23 in June to 3.85 in October indoors and from 0.63 in September to 6.83 in October outdoors and cattle biting density from 4.81 in May to 191.14 in August.

In all areas except one and in all months except May the resting density was higher in cattle sheds than in human habitations and outdoors. Cattle sheds accounted for 58.7% of the resting population, human habitations for 27.05% and outdoors for 14.25%. The average resting density in human habitations, outdoors and in cattle sheds was 3.44, 6.53 and 14.17 respectively.

The species was found to be highly zoophilic. Out of the total biting mosquitoes 97.3% were collected on cattle and only 2.7% on humans. Among human biting mosquitoes 38.1% prefer to feed indoors and 61.9% outdoors. The average biting density on man was 1.27 indoors and 2.06 outdoors and 59.46 on cattle. Further studies are in progress.

6.7. Studies on Musca Domestica

The common house fly *Musca domestica* Linnaeus is the subject of routine studies at the VCRC mainly for the purpose of undertaking control measures using parasitoids and juvenile hormone compounds.

The density of *M. domestica* in the study area is being monitored both in indoor and outdoor by using Scudder's grill method in which flies sitting in the grill for a time interval of 30 seconds are recorded. The number of flies resting in indoor and outdoor range from 0 to 13.4 per grill and 0 to 2.8 per grill respectively.

Immature density in the manure heaps of the study farms are monitored by litre sampling method at weekly intervals. The manure collected randomly at different points of the manure heaps is brought to the laboratory. The larvae are hand picked and the density of different instars are recorded while the puparia from the samples are isolated by employing floating and skimming methods and kept for the possible emergence of either flies or parasitoids. The density of larvae ranges from 0 to 601 per litre and pupae from 0 to 724 per litre. Further studies are in progress.

IV. MISCELLANEOUS

1. Educational Programme

The first batch of 8 M.Sc. Medical Entomology students have passed out in May, 1988. All of them have got placements in different National Institutes/Organizations. There are 9 students in the II M.Sc. who have completed most of their course work and will be submitting their Dissertations shortly, on the followingTopics:

1. Studies on the degradation of Fenthion by the Bacteria from Cess Pits.
2. Physico-Chemical factors affecting the Breeding and Abundance of *Culex quin quefasciatus* in Pondicherry
3. Studies on *Anopheles subpictus* complex.
4. Studies on Predation potential of some insect predators of Mosquito larvae.
5. Evaluation of different compounds for Oviposition attractancy against *Culex quinquefasciatus.*
6. Evaluation of Deltamethrin and Cypermethrin against Mosquitoes, House flies, Cockroaches and Bedbugs.
7. The susceptibility status of Mosquitoes (Vectors) to Pesticides used in Public Health programmes in Thiruvannamalai of North Arcot Dt. (Tamil Nadu)
8. Studies on the susceptibility of *Romanomermis iyengari* to selected Pesticides used in the Paddy field.
9. Role of Juvenile harmones in Controlling House flies, *Musca domestica* L. (Diptera: Musidae).

Eleven students were admitted for the 1988–89 academic year. Among them, 6 are fresh graduates, selected by open competition, 4 are in-service candidates, and one Ghana national is the WHO sponsored candidate.

The 3 Research Fellows selected during the year have registered for Ph.D. in the disciplines of Microbiology and Genetics, raising the total number of Ph.D candidates working in the Institute to 18.

The following visiting Scientists delivered Guest lectures to the P.G.Students during the year.

1.	Dr. V. Dhanda Director Grade Scientist NIV, Pune	*Ticks, Mites and Leishmaniasis*
2.	Dr. M. K. Goverdhan Deputy Director NIV, Pune	*Viruses*
3.	Dr. M. A. Srinivasan Deputy Director, NIV, Pune.	*Electron Microscopy and Rodents*
4.	Dr. Rene Le Berre WHO, Geneva.	*Onchocerciasis and Trypanosomiasis*
5.	Dr. Mir S. Mulla Prof. of Entomology University of California U.S.A.	*Integrated Control of stagnant water mosquitoes*
6.	Dr. Harold C. Chapman Executive Director of AMCA U.S.A.	*Pest nuisance and mosquito abatement*

7.	Dr. K. M. Rao Head, Entomology and Bio-chemistry Division DRDE, Gwalior.	*Schistosomiasis*
8.	Dr. B. V. Rao, Prof. Deptt. of Parasitology College of Veterinary Science, Tirupati	*Zoonotic Helminthic diseases and Zoonotic protozoan diseases*
9.	Dr. Brian Kay Chairman: Parasit/Ento Qd. lust Med. Research Brisbane	*Aedes control*
10.	Dr. Mostafa Hussein Harb Director Malaria/Filariasis Egypt	*History of malaria and filariasis control in Egypt*
11.	Dr. Mikhail Mikhail Matta Director Endemic Diseases, Egypt	*Epidemiology and control of Bilharziasis*
12.	Dr. D. S. Choudhury Deputy Director Malaria Research Centre New Delhi	*Malaria*

2. MEETINGS/CONFERENCES/SEMINARS SYMPOSIA ATTENDED

Dr. P.K. Rajagopalan, Director attended a seminar on Japanese Encephalitis at the School of Tropical Medicine, Calcutta on 8th April 1988. He attended the XII International Congress for Tropical Medicine and Malaria and took part in the Round Table Session on water supply and sanitation, held at Amsterdam from 18th to 23rd September 1988. He attended the Steering Committee Meeting of the Scientific Working Group on Filariasis from 26th to 28th September 1988. He participated in an Informal Consultation meeting on Bacterial formulation for cost effective vector control in endemic areas from 19th to 21st October 1988 and was a coopted member at the Biannual meeting of the Steering Committee on Biological Control of Vectors, from 24th to 28th October 1988, held at Vector Control Research Centre, Pondicherry, organized by the WHO Special Programme for Research and Training in Tropical Diseases, WHO, Geneva. He also chaired a session at a symposium on 'Significant Advances in Vector Control with special reference to Malaria' held at New Delhi on 22nd November 1988, organized by M/s. Roussel Pharmaceutical (India) Limited.

As a member of the Governing Body of the Indian Council of Medical Research, he attended the meetings held on 16th April and 8th June 1988 at New Delhi. He also attended the six monthly review committee meeting of the Scientific Adviser to the Prime Minister, on Science and Technology Mission Project on Integrated Vector Control of Malaria, Filaria and other vector borne diseases on 8th July at New Delhi. He continues to be the member of the Academic Council and Planning Board of the Central University, Pondicherry and attended the meetings on 30th August and 10th November 1988 respectively.

He is a member of the Scientific Advisory Committee of the National Institute of Virology, Pune. He continues to be the member of the Technical Advisory Committee on Malaria and a member of the Review Committee on Urban Malaria Scheme, constituted by the Government of India, Ministry of Health and Family Welfare, New Delhi, of the Working Group on Evaluation

of the application on the use of Remote sensing Techniques, nominated by the Government of India, Ministry of Health, of the Governing Council of the Salim Ali School of Ecology, Central University, of the Environment Sub Group on Narmada Control Authority; and, a member of the State Planning Advisory Committee of the Planning and Research Department, Government of Pondicherry.

Dr. P.K. Das, Deputy Director, attended the Symposium on "A significant advance in Vector Control with special reference to Malaria", at New Delhi, on 22nd November, 1988.

Dr. K. Balaraman, Assistant Director, participated in the (i) National symposium on "Recent Trends in Biotechnology" held at Trivandrum in June, 1988. (ii) "Third National Congress of Veterinary Parasitology" held at Tirupati, in November, 1988. (iii) "Informal Consultation on Bacterial Formulations for Cost-effective Vector Control in endemic areas" Organised by the WHO, at VCRC Pondicherry, in October, 1988. He has been sponsored as a member of the "American Mosquito Control Association, Inc."

Dr. K.N. Panicker, Assistant Director, attended the Review meeting of the "Technology Missions" at Planning Commission, Delhi, in December, 1988. He was a resource person for a UNICEF sponsored workshop for N.S.S. Programme officers, at Pondicherry, held in September, 1988.

Dr. S.P. Pani, Assistant Director, is the member of Indian Association of Communicable Diseases and the Indian Public Health Association.

Mr. K.D. Ramaiah, attended the symposium on "Filariasis" organised by the Academy of Medical Sciences, Annamalai University and National Academy of Medical Sciences, New Delhi, at Annamalai University on 16.7.1988. He also attended the National seminar on "Japanese Encephalitis" organised by the Govt. of Andhrapradesh at Hyderabad, on 30.8.88.

Mrs. Nisha George participated in the workshop on "Inhalation Toxicology" and a symposium on "Cellular and Molocular Toxicology" held at Defence Research and Development Establishment, Gwalior, from 7th to 10th September 1988.

Miss. M. Jayashree, attended the second National symposium on "Fish and their environment" held at Trivandrum from 21st to 28th November, 1988.

3. LIBRARY

About 250 books, 8 journals and 500 reprints pertaining to malaria, filariasis, vectors and their control were procured from national and international agencies and added to the library during the period under report.

The holdings of the library as on 31.12.1988:

Books	2740
Bound Journals	1217
Journals	84
Annual Reports	115
Video Casettes	6
Reprints	2500

All reprints were indexed subject wise with the aid of computer for easy retrieval by the readers As usual the library continues to provide bibliographic service, current awareness service, reference service, reprint request service, inter library loan service to the students, researchers and trainees. Selected papers and reprints were periodically sent to the field stations for current awareness of the staff stationed there. Several scientists from other institutions availed the facilities provided by the library.

4. INFORMATION RETRIEVAL SYSTEM

The Information Retrieval System continues to update the recent and past references on all aspects of Vector Biology and Control. This system has now switched over to the more advanced software in order to sort and retrieve data much faster than the existing programs. This system also enables all the subjects to be integrated and retrieved through the use of keywords. All the faculty members and students involved in independent research for their Ph.D. and M.Sc. courses are utilising the existing IRS facility of the centre. IRS system banks mainly on the momentum

and interest of all concerned scientists of the centre to share the information they have independently gathered, so as to be utilised by all.

The Computer division has been further expanded with the acquisition of 1 PC/AT 286 and 1 PC/AT 386 Computers apart from 9 discless nodes. All the Computers have been networked and existing peripheral hardwares and softwares can be simultaneously used from several points within the centre.

5. TRAINEES DURING THE YEAR

1. Ms. Aparna Chakravorty, Tripura University, Agartala, Tripura.
2. Mr. Bhaskar C. More, Deptt. of Zoology, University of Pune.
3. Mr. M. de Bruyne, Deptt. of Entomology, Wageningen, Agricultural University. (WHO Placement).
4. Mr. Eswaraiah, Rishivalley School, Andhrapradesh.
5. Dr. N. Elangeswaran, Assistant Director, Central Leprosy Teaching and Research Institute, Chinglepet.
6. Mr. Khairnar. G.J., Deptt. of Zoology, University of Pune.
7. Dr. Mostafa Hussein Harb, Director, Malaria /Filariasis, Egypt. (WHO Placement).
8. Dr. Mikhail Mikhail Matta, Director, Endemic Diseases, Egypt. (WHO Placement).
9. Mr. P.V. Mohammad Ismail, Asst. Entomologist, Directorate of Medical & Health services, Union territory of Lakshadweep.
10. Mr. N. Ninge Gowda, Research scholar, Deptt. of studies in Zoology, Manasagangotri, Mysore.
11. Mr. M. Rajendran, Central Leprosy Teaching and Research Institute, Chinglepet.

6. VISITORS DURING THE YEAR

1. Dr. J. Akiyama, WHO/SEARO, Delhi.
2. Dr. Amarat Bhumiratna, Mahidal University, Bangkok, Thailand.
3. Dr. S.S. Agarwal, Sanjay Gandhi P.G. Institute of Medical Sciences, Lucknow.
4. Prof. T.N. Ananthakrishnan, Director, Entomology Research Institute, Madras.
5. Dr. O.P. Barghava, Sri Ramachandra Medical College, Madras.
6. Dr. Bryan T. Grenfell, University of Sheffield
7. Dr. Bruce Knudsen, WHO/VBC, Geneva.
8. Dr. M. Bhandari, Sanjay Gandhi P.G. Institute of Medical Sciences, Lucknow.
9. Dr. Bala N. Devisetty, Abbott Labs, U.S.A.
10. Dr. Bampat Napompeth, Kesetsart University, Bangkok, Thailand.
11. Dr. Boris Dobrokhotov, WHO/TDR, Geneva.
12. Dr. Brian Kay, Qd. lust, Medical Research, Brisbane.
13. Dr. D.K. Cahabra, Sanjay Gandhi Institute of Medical Sciences, Lucknow.
14. Dr. Christopher Aly, Germany.
15. Dr.D.S. Choudhury,DeputyDirector,M.R.C., New Delhi.
16. Dr. Christine Dahl, Uppsala University, Sweden.
17. Dr. Donald A. P. Bundy, Director, Imperial College, London.
18. Dr. V. Dhanda, NIV, Pune.
19. Dr. David L. Madden & Mrs. Nancy R. Madden, American Embassey, New Delhi.
20. Dr. M.K. Goverdhan, Deputy Director, NIV, Pune.
21. Dr. M.J. George, PFCP, Delhi.
22. Dr. Harold C. Chapman, Executive Director of AMCA, U.S.A.
23. Dr. Jiri Vavra, Charles University, Prague, Czchoslovakia.
24. Dr. Jose Duarte de Araujo, National Research Council, Brazil.
25. Dr. Klier, Instt. Pasteur, Paris.
26. Mr. S.M. Khaire, Sandoz (India) Ltd., Bombay.
27. Mr. V. Krishnamurthy, Sec. to Govt. of Kerala, Health & Family Welfare Deptt.
28. Dr. L. Lacey, VBC/AID, U.S.A.
29. Dr. R.C. Mahajan, Prof., Department of Parasitology, PGI, Chandigarh.
30. Dr. L. N. Mohapatra, Director, RMRC, Bhubaneshwar.
31. Dr. Mir S. Mulla, Prof. of Entomology, University of California.
32. Dr. Martin A. Odei, Instt. of Aquatic Biology, Achimota, Ghana.
33. Prof. M.K.K. Pillai, Delhi University, Delhi.
34. Dr. Piene Guillet, WHO, Generva.
35. Dr. Peter Luthy, Instt. of Microbiology, Switzerland.

36. Dr. P.K. Ramachandran, DRDE, Gwalior.
37. Dr. N. Ramakrishnan, National Fellow, Division of Entomology, IARI, New Delhi.
38. Prof. K.M. Rao, Deputy Director, DRDE, Gwalior.
39. Dr. B.V. Rao, Prof. of Parasitology, Veterinary College, Tirupati.
40. Dr. Ries de Winter, Amsterdam.
41. Dr. Rene Le Berre, WHO, Geneva.
42. Dr. N. Rishikesh, WHO, Geneva.
43. Dr. V.P. Sharma, Director, MRC, New Delhi.
44. Dr. B. Speight, Shell Research Ltd., U.K.
45. Dr. Sushil K. Khetan, HIL Res. & Devpt. Centre, Haryana.
46. Dr. S. Sundarajan, Deputy Director, FIPPAT, Padappai.
47. Prof. M.G.R. Varma, Prof. of Entomology, London School of Tropical Medicine and Hygiene, London.
48. Dr. Xu Bozhao, Instt. of Parasitic Diseases, China.
49. Dr. H.H. Yap, University Sains Malaysia.

7. PAPERS PRESENTED AT CONFERENCES/MEETINGS/SYMPOSIA

1. **P.K. Rajagopalan & K.D. Ramaiah.**
   Vectors of Japanese encephalitis in India, their breeding habitats and control.
   Proceedings of the seminar on "Japanese Encephalitis", West Bengal, 1988.

2. **P.K. Rajagopalan, P.K. Das & K.D. Ramaiah.**
   Vectors of Filariasis and their control.
   Symposium on "Filariasis", Annamalai University, 16th July, 1988.

3. **K. Balaraman.**
   Bacillus thuringiensis H 14 mutant with potential for producing Entomotoxin and Dihydroxy Phenylalanine.
   National symposium on "Recent trends in Biotechnology" held at Trivandrum, in June 1988.

4. **K. Balaraman.**
   Prospects of Biological Control of Vectors.
   "Third national Congress of Veterinary Parasitology" held at Tirupati, in November 1988.

5. **K. Balaraman.**
   Review on Microbial Formulations for Vector Control.
   "Informal Consultation on Bacterial Formulations for Cost-effective Vector Control in endemic areas" conducted by the WHO at VCRC Pondicherry, in October, 1988.

6. **M. Jayashree**
   Culture and conservation of Giant gourami, (*Osphronemus goramy*, Anabantoidei) an ideal food fish and a Bio-control agent for aquatic weed control.
   Second National symposium on "Fish and their Environment" held at Trivandrum from 21st to 28th November, 1988.

8. LIST OF PUBLICATIONS

1. **N. Pradeep Kumar, S. Sabesan, M. Kuppusamy and K. Balaraman.**
Effect of controlled release formulation of *Bacillus sphaericus* on Mansonia breeding.
IJMR 87, 1988, 15–18.

2. **D. Amalraj, V. Vasuki, C. Sadanandane, M. Kalyanasundaram, B.K. Tyagi and P.K. Das.**
Evaluation of two new juvenile hormone compounds against mosquito vectors.
IJMR 87, 1988, 19–23.

3. **P.K. Rajagopalan and P.K. Das.**
What ails mosquito control programmes in India?
Bulletin of Sciences, 4(1), 1988, 14–17.

4. **D. Amalraj, V. Vasuki, M. Kalyanasundaram, B.K. Tyagi and P.K. Das.**
Laboratory and field evaluation of three insect growth regulators against mosquito vectors.
IJMR 87, 1988, 24–31.

5. **P.K. Rajagopalan, P.K. Das, S.P. Pani, T. Mariappan, A.R. Rajavel, K.D. Ramaiah, D. Amalraj, K.P. Paily, N. Balakrishnan, C. Sadanandane, P. Vanamail, S. Subramanian, R. Srinivasan, N. Arunachalam, C.M.R. Reddy, C.B.S. Reddy and N. Somachary.**
Evaluation of integrated vector control measures on filariasis transmission in Pondicherry.
IJMR 87, 1988, 434–439.

6. **Vectrol Control Research Centre.**
Mosquito control master plan for Bangalore city.
ICMR Bulletin 18(5), 1988, 43–47.

7. **S. Cairncross, A.R. Rajavel, P. Vanamail, S. Subramanian, K.P. Paily, K.D. Ramaiah, D. Amalraj, T. Mariappan and R. Srinivasan.**
Engineering, mosquitoes and filariasis: a case report.
J. Trop. Med. & Hyg. 91 (3), 1988, 101–106.

8. **P.K. Rajagopalan**
Insects, communicable diseases and health role of government, society and individual
Swasth Hind, 32,(3) & (4,) 1988, 78–81.

9. **P.K. Rajagopalan.**
Malaria and its control in India.
Health for Millions, Aug. 1988, 6–8.

10. **P.K. Rajagopalan and P.K. Das.**
Primary health care: Theory and practice in the Indian context.
ICMR Bulletin 18, (6), 1988, 55–59.

11. **K. Balaraman and S.L. Hoti**
Comparative cost of mosquito control with larvicidal Bacilli and insecticides.
The Environmentalist 8, 1988, 123–126.

12. **C.H. Sujatha, V. Vasuki, T. Mariappan, M. Kalyanasundaram and P.K. Das.**
Evaluation of plant extracts for biological activity against mosquitoes.
International Pest Control, 30(5), 1988, 122–124.

13. **A.M. Manonmani, V. Vasuki and K. Balaraman**
Establishment of a standard test method for determining susceptibility of mesocyclops to different insecticides.
IJMR 89, 1989, 42–46.

14. **P. Jambulingam, K. Gunasekaran, S.S. Sahu, P.K. Hota, B.K. Tyagi and M. Kalyanasundaram.**
Effect of permethrin impregnated bednets in reducing population of malaria vector *Anopheles culicifacies* in a tribal village of Orissa State (India).
IJMR 89, 1989, 47–50.

15. **N. Pradeep Kumar, S. Sabesan and K.N. Panicker.**
Biting rhythm of the vectors of malayan filariasis, *Mansonia annulifera*, *M. uniformis* and *M. indiana* in Shertallai, Kerala State.
IJMR 89, 1989, 51–54.

VCRC PUBLICATIONS

1. Mosquito
2. Control of Brugian filariasis in Shertallai, South India: pre-control epidemiological observations.
3. Filariasis: A training module for middle level worker.

AWARDS

Dr. P.K. Rajagopalan was awarded a Gold Medal instituted by **Charles University, Prague, Czechoslovakia**, for his long standing contributions to Vector Biology Research. The Medal was presented by Dr. Jiri Vavra at a function at The VCRC on 25th October 1988.

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